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# WASTE TREATMENT PROTOZOA FLAGELLATA

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1962

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# WASTE TREATMENT PROTOZOA
## FLAGELLATA

WILSON T. CALAWAY and JAMES B. LACKEY

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This report offers persons engaged in the operation of waste treatment works an overview of some of the problems to expect. General topics covered include: sampling and handling of protozoa and laboratory equipment; examination and classification of the flagellates of sewage treatment processes; and specific classes. Solutions used in microscopy are described. A glossary and bibliography are included.

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# WASTE TREATMENT PROTOZOA

## FLAGELLATA

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**1962**

COLLEGE OF ENGINEERING
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# PREFACE

One of the important specialty areas in which research studies are performed under the aegis of the College of Engineering at the University of Florida, is concerned with environmental engineering at the Earle B. Phelps Laboratory for Sanitary Engineering Research. Projects are selected which will contribute to improvements in two of Florida's most valuable assets; namely, its important air and water resources which are so essential for continued progress within the State.

The kind of research that is undertaken requires the services of chemists and microbiologists as well as engineers. Indeed, most of the basic research in environmental engineering has been done by these professional groups, and this publication represents the culmination of over ten years of work by Professor Wilson T. Calaway.

One of the principal differences between engineer and non-engineer scientists is that engineers are generally more concerned with the utility or possible practical applications of research results. However, Dr. Lackey is a microbiologist who is competent on practical as well as basic research, and he has demonstrated penetrating introspection in searching for functional uses of newly acquired information. This contribution should be welcomed by persons engaged in the operation of waste treatment works since it will give them a clearer picture of some of the things they will encounter.

John E. Kiker, Jr.
Professor of Civil Engineering
Head, Sanitary Engineering Section

# ACKNOWLEDGEMENTS

Many persons have contributed toward the completion of this book. Since large sections of Pascher had to be translated, the help of Mr. Emile Wenzel, who was at that time operator at the University of Florida Sewage Treatment Plant, was enlisted. Mr. Wenzel gave freely of his time in translating many passages and in aiding with difficult portions of material which one of us translated. Special appreciation must be expressed to him for this work. The following have been kind enough to read the manuscript and make suggestions: Dr. E. G. Pringsheim, Dr. Eugene C. Bovee, Dr. Richard B. Vowles, and Mrs. Jacquelyn Hynes. Appreciation is expressed to Professor Ralph W. Kluge, Head of the Department of Civil Engineering, and to Professor John E. Kiker, Head of the Sanitary Engineering Section, for their patience and encouragement. Finally, to Mrs. Anna Jo Calaway, for her many services of typing, correcting and assistance in rewording, we wish to express a very special "Thanks."

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# Waste Treatment Protozoa

## PART I

### SAMPLING AND HANDLING

### INTRODUCTION

In recent years there has been an increased tendency for engineers to include laboratory facilities when designing waste treatment works and to specify laboratory apparatus just as they would pumps and other equipment. A microscope is frequently included as a part of the working equipment of the laboratory because of a realization on the part of engineers that it can be a useful tool in process control.

The operator who has access to a microscope can well take advantage of this instrument to become acquainted with some of the myriad true laborers in waste treatment processes. These workers are (1) bacteria and fungi, (2) protozoa, (3) algae and (4) metazoa. Bacteria are the primary laborers but, with the exception of *Zooglea ramigera* and some related forms, iron bacteria, sulfur bacteria and nitrifying bacteria, little is known of many other bacteria of sewage treatment or the part they play. Unfortunately microscopic examination yields little information concerning most bacteria because of their minute size and lack of differences. Their study requires culturing and testing and, by the time results can be obtained, the conditions which produced the bacterial flora under study may have been superseded by other conditions. While microscopic study does reveal the presence of certain forms, it usually tells little about their activity because many bacteria are tolerant of adverse conditions and can be present though not thriving.

Algae which contain chlorophyll are of little importance in sewage treatment except on the surface of trickling filters. However, the use of oxidation ponds is rapidly increasing and, in these, algae are of great importance. Some of the flagellated green forms are classified as protozoa by many zoologists.

The single-celled animals (or protozoa) and the higher forms (or

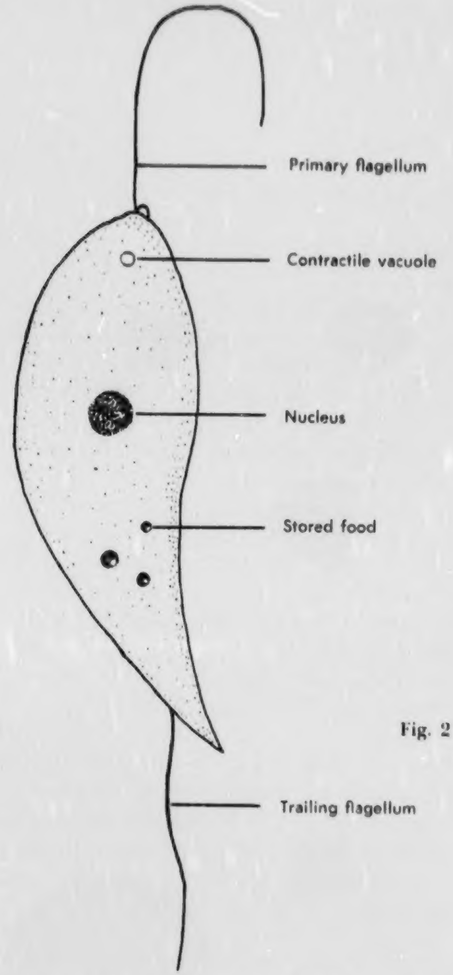
Primary flagellum
Contractile vacuole
Nucleus
Stored food
Trailing flagellum

Fig. 1 An Animal Flagellate (*Bodo caudatus* much enlarged)

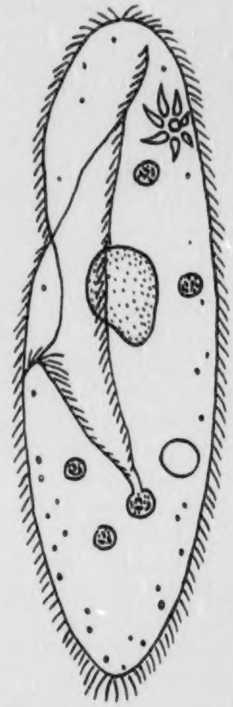

Fig. 2 A Ciliate—*Paramecium caudatum*

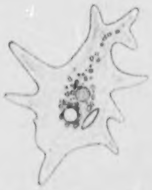

Fig. 3 An Amoeba—showing pseudopodia

metazoa) can, however, yield considerable information by direct microscopic examination. If they are active, then the conditions present are satisfactory for the organisms observed. The metazoa are usually the largest of the forms present in waste treatment processes. Generally speaking, the most numerous metazoa are rotifers, with nematodes, bristle worms (oligochaets) and turbellarians following in that order. Included in the metazoa are, of course, the insects and their larvae which also play an important part in waste treatment processes. The metazoa include many diverse groups about which the information is of a general nature only, and they will not be discussed at this time.

## PROTOZOA

There exists, however, a fair amount of information concerning the protozoa—a group which is of particular interest to the sewage plant operator. The protozoa associated with waste treatment belong to three groups of one-celled animals—namely, flagellates (Mastigophora), amoebas (Sarcodina), and ciliates (Ciliata). Each group has a characteristic means of movement. The flagellates (see Fig. 1) move by means of long hairlike devices which, in the organisms associated with waste treatment, number from 1 to 8. The *flagella* (whips) are usually ½ to 1½ times as long as the animal they serve. In almost all cases, the *flagella* pull the organisms. The ciliates (Fig. 2) are furnished with numerous short hairlike devices known as *cilia* (eyelashes). These are only occasionally as long as the greatest width of the animal and may be present all over the body or in very limited areas. The amoebas (Fig. 3) move by extending a portion of the body known as a *pseudopodium* (literally false foot) by a kind of flowing action, after which the whole body may flow into the *pseudopodium*. Occasionally, the entire organism may move with a flowing-creeping-rolling action.

## THE MICROSCOPE AND ITS ACCESSORIES (FIG. 4)

The microscope ordinarily furnished for the waste treatment plant laboratory is a monocular microscope with a triple nosepiece. The ocular or eye lens is usually ten power (10X). The objectives (the lenses mounted on the revolving nosepiece) are usually about ten power (10X), forty power (40X) and ninety power (90X). These nosepiece lenses with the 10X eye lens will give magnifications of 100, 400 and 900. The highest power is used with oil between the cover glass or

Fig. 4 Microscope Bench (This is Research Equipment—Simpler Equipment May Be Used)

specimen and the objective and finds its principal use in examination of bacteria. Oil is used because without the oil so much light is lost at this high magnification that the specimens are almost impossible to see. The two lower powers are the most useful for examining protozoa, but occasionally the oil immersion must be used for fine structures. When oil immersion is used for examining protozoa, a thin cover glass (No. 1) must be used. Flagella are difficult to see and the location of cilia is difficult to determine, but opening or closing the substage diaphragm and raising or lowering the light focusing lens under the microscope stage may make these structures more apparent. Light coming from one side and practically outside of the visible field also increases the ability to discern flagella and cilia. Such oblique lighting may be obtained by partially covering the bottom opening of the substage lens (the diaphragm must be wide open).

A fine binocular microscope with phase lenses will make examination of protozoa much simpler. The binocular microscope may be used with less eye strain, and the phase equipment allows ready observation of flagella, cilia and other structures normally very difficult to see. Very active organisms, however, present problems in observation even when the finest optical equipment is available.

If counting of organisms is to be done, and this is frequently desirable, the microscope must be equipped with a mechanical stage. Estimates of sizes of organisms are also useful in identification. Therefore, it is important that the eyepiece be fitted with a ruled scale known as an ocular micrometer. The linear type scale is preferable but a Whipple type can be made to serve. A good microscope illuminator is a real asset.

### MICROSCOPIC EXAMINATION—PREPARATION OF THE SAMPLE

The material to be examined can be activated sludge, mixed liquor or return sludge, scrapings from a trickling filter at about the 1 to 1½ ft. depth, washings from the sand of an intermittent sand filter, bottom sediment from a receiving stream or a sample of the water of the stream concentrated by centrifuging. Less informative but worthy of study is secondary sludge from trickling filters and digesting sludge. The latter is commonly almost devoid of protozoa but this may not be true under certain conditions, such as those sometimes found in uncovered digesters. Oxidation ponds (sewage lagoons) always have an interesting biota both in the bottom sludge and in the overlying water.

If the material to be examined is not homogeneous, it may be homogenized by being forced back and forth through a pipette. The pipette used for handling the sample is made by drawing down a piece of 6 to 8 mm tubing so that the tip opening is approximately 1½ mm in diameter. The tubing is cut to yield a pipette 25 cm (10 in.) long and is equipped with a soft rubber bulb of 2 ml volume.

After the sample is thoroughly mixed, one drop of it is allowed to fall on a microscope slide with the tip of the pipette about 1 cm above the slide. Over this is then dropped a cover slip (preferably No. 1, 25 mm x 25 mm). The sample is now ready to examine.

The Sedgwick-Rafter cell may also be used but in the case of sludges dilution is necessary. High power objectives cannot be used with this cell. This is a distinct handicap in examining protozoa, particularly the minute flagellates, and for this reason the drop-cover slip method is preferred.

### AIDS TO MICROSCOPIC EXAMINATION

In order that the relation of the protozoa to treatment may be determined, it is usually necessary to identify the organism. This is a two-way street, since identification allows the sewage plant operator to determine what others have already discovered about the organism at hand, and also allows him to increase his fund of knowledge by recording physical and chemical conditions and associated protozoa and metazoa. Such information may in the future be very valuable.

Identification is dependent upon shape, size, and upon the presence and arrangement of certain structures. The shape and size are best determined on living animals. Because of motion, the number and arrangement of flagella and the arrangement of cilia can often be determined only by stopping or retarding the movement of the animal, or by coloring the structures by dyes or stains. Stains are perhaps even more important in determining the location and form of the nucleus of the cell and certain related structures known as micronuclei and kinetonuclei.

Four solutions are especially helpful because of their ease of use. Each solution has a specific use, and the formula for each is listed in Appendix A.

1. Noland's Stain

This stain kills the protozoa and at the same time stains the flagella and cilia. It is useful for determining the number and arrangement of flagella and the arrangement of cilia. It also stains nuclei, but not very satisfactorily since they are obscured by stained structures on the surface of the animal. It is commonly used by mixing one drop of the stain, either as given or after dilution, with a drop of the material to be examined before adding the cover slip. Unfortunately, small flagellates often become "debris" when handled in this manner and it is preferable to add the stain (which may be diluted 1 to 5 or more) to the edge of the cover slip. This is best done with a finely drawn pipette. The flagellate of interest should be watched as the dye overtakes it. This method requires careful operation of the mechanical stage since the addition of liquid to the edge of the cover slip causes rapid flowing of the liquid under the cover.

2. Methyl Green—Acetic Acid

This stain also kills and stains simultaneously. It stains nuclei, micronuclei, kinetonuclei and perhaps some related structures a bright green. If the extraction (see formula) has been carefully done, no further staining of the cell will occur. The stain is used by mixing one drop of it with one drop of the material to be examined, or better, by running the dye under the cover slip as recommended for Noland's Stain.

3. Neutral Red—A Vital Stain

This dye stains certain structures specifically. It is also an acid base indicator and shows up as a bright cherry red color (acid) or as a yellow color (base) in food vacuoles. Other vacuoles are also colored by this dye and it stains the nucleus slightly. It is particularly important as a stain for granules in certain euglenas.

One drop of the working solution of the dye is added to one drop of the material to be examined. This is done on a slide before covering with a cover slip. The dye and alcohol strength of the working solution (0.02% and 2% respectively) do not adversely affect, or only slowly affect, most organisms. They can, therefore, be examined in the living state.

4. Methyl Cellulose

This material yields a very viscous solution which is used by placing a small ring of methyl cellulose on a slide (the ring diameter should be about ½ to ⅔ the cover slip width), and placing a small drop of the biological material in the center of the ring. The cover slip may then be added. This effectively slows down organisms so that flagella and cilia, and also other structures, may be readily observed.

One further technique will be found to yield additional information. In this method the prepared slide is placed in a wet chamber and allowed to stand for a period of time. The wet chamber consists of a petri dish with several filter papers cut to fit in the bottom of the lower dish. The filter paper is moistened with as much distilled (*not tap*) water as it will hold. The slide is then laid face up on the moist filter paper and the dish covered with its top, thus preventing evaporation from the slide. The prepared slide may be held for 2½ to 24 hours or more and the changes will be somewhat dependent on the holding time. The most noticeable effect of holding is that the center beneath the cover slip becomes anaerobic, and motile forms of both bacteria and protozoa seek their preferred oxygen level. The various levels gradate inward from the edge, and a noticeable feature is a line of bacteria a short distance from the edge. The location of protozoa may be in part conditioned by the presence of bacteria as food or, in the case of predatory protozoa, the presence of other protozoa which they may consume. Feeding habits may often be observed by this simple method. It frequently brings about the retardation of movement of very active forms, and other forms such as amoebas begin to move about and are more readily observed. Changes in number occur under such conditions and, if holding times are long, some species which were present only in small numbers may become dominant, while those that are most numerous at the time of collection may disappear. Some organisms are widely tolerant of oxygen deficiency and may be seen near the middle of the slide as well as near the edge. If they are more numerous near the edge, it may be because their food is more concentrated there.

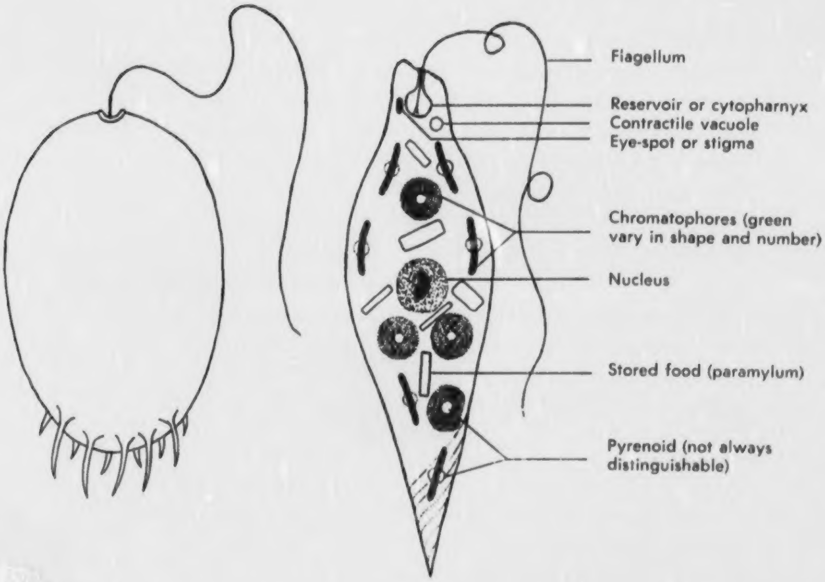
Flagellum
Reservoir or cytopharnyx
Contractile vacuole
Eye-spot or stigma
Chromatophores (green vary in shape and number)
Nucleus
Stored food (paramylum)
Pyrenoid (not always distinguishable)

Fig. 5 Euglenoids (schematic)
a. A *Trachelomonas*—an armored flagellate
b. A *Euglena*—one of the naked flagellates

# PART II

## THE FLAGELLATES OF SEWAGE TREATMENT PROCESSES

Of the protozoa associated with sewage treatment, the group or subphylum embracing the flagellates has more species represented than do either of the other two subphyla. More than 125 species are known to exist and at times thrive in trickling filters, activated sludge, polluted streams and similar environments. They are likely to be more numerous under anaerobic or near anaerobic conditions, but some are little affected by this aspect of their environment while others are found only when conditions produce good aerobic treatment. Since many of them are quite small and very active, their examination may challenge the skill of the operator in making microscopic examinations, but careful study can be very rewarding. The operator should soon be able to recognize some common forms and formulate ideas concerning their significance.

### MICROSCOPIC EXAMINATION—WHAT TO LOOK FOR IN FLAGELLATES

1. *The Flagella* (see Figs. 1, 5 and 6)

The number of flagella, their size, their point of origin and their normal position relative to the organism and to each other (if more than one is present) are all significant. The action of the flagellum or flagella in swimming is also important and will be more thoroughly discussed under "Motion." The number of flagella is often difficult to determine, particularly if more than one active flagellum is present. Extreme caution must then be exercised to avoid premature conclusion that only two are present. Usually the flagella arise from the forward (anterior) end but occasionally they originate laterally (from the side), and there is a small but important group in which the flagella emerge from both sides (see Figs. 141-149). In some organisms the flagella originate at the anterior tip (apex) but opposite one another and at an angle to the body axis of the organism (see Figs. 33 and 35). Trailing flagella are usually inactive and are difficult to observe, especially if very fine or if they are sometimes hidden by the body. In a few small groups such flagella adhere to the body. Flagellar sizes are usually reported relative to body length. Regardless of its position on the body, a flagellum is rooted in a basal granule or blepharoplast just under the body surface. This cannot be seen except by special staining procedures, but two related arrangements can be distinguished. The blepharoplast is occasionally associated with a larger dense structure known as a parabasal body and the two together are known as a kinetonucleus. This may sometimes be seen in direct microscopic examination when the observer has had considerable experience in looking for it. Flagella may also arise from the cell nucleus which is moved well forward but is usually deeper than the kinetonucleus would be. The worker may be aided in the examination of flagella by Noland's Stain and in the determination of the nucleus and kinetonucleus by methyl green-acetic acid, both of which were discussed in Part I.

### 2. *Size* (see also Appendix B)

Size varies considerably between species and to some extent between individuals of the same species. The smallest flagellates associated with waste treatment are about 5 microns ($\mu$) in length ($1000\mu = 1$ mm; 25 mm = 1 in. or $25{,}000\mu = 1$ in.) and the largest may be almost $500\mu$ (*Euglena oxyuris*) or about ½ mm. Flagellated protozoa as large as $500\mu$, which are associated with sewage treatment, will ordinarily be found only in polluted streams. Flagellates found in the sewage treatment processes themselves will rarely exceed $100\mu$. The length is estimated by the ocular micrometer, which should be calibrated by use of a stage micrometer. Care must be taken in interpreting size. If, for example, the description states that the organism is $17\mu$ long, this is probably the average length. Organisms $8\text{-}9\mu$ long and those $25\mu$ in size should not be dismissed as not belonging to the species if they agree in other characteristics since considerable range in size is common. The ratio of length to width is usually a dependable characteristic but normal overfed organisms may be distorted and vary in length as well as in the ratio of length to width.

### 3. *Shape or Morphology*

Shape descriptions vary considerably according to interpretations of the describers. Even exceeding this source of variability is the variability of most organisms themselves. This is most apparent in the *Mastigamoebae* which appear to be amoebas with flagella and, when crawling or gliding on a surface, exhibit a con-

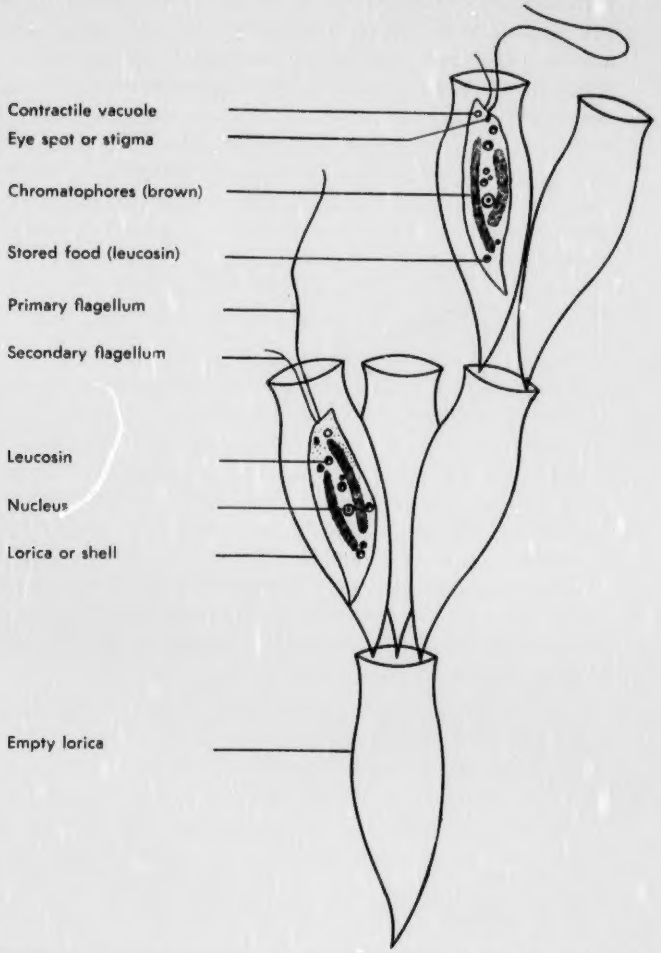
Contractile vacuole
Eye spot or stigma
Chromatophores (brown)
Stored food (leucosin)
Primary flagellum
Secondary flagellum
Leucosin
Nucleus
Lorica or shell
Empty lorica

Fig. 6 A Colonial Plant Flagellate—*Dinobryon sertularia*—(many times enlarged)

stantly changing form that cannot be readily described. When they swim their shape is somewhat more characteristic and more like that of flagellates.

At the other extreme from these very changeable forms are species with rigid bodies often showing helical twisting, grooves, keels or similar structures. There are also forms that dwell within shells; their apparent shape is the shape of the shell (see Appendix B). Other forms have delicate collars which stand out from the body and usually surround the base of the flagella, and still others have spines and some possess ribbing or sculpturing. Overfed organisms may be distorted in shape as well as in size. Environmental conditions appear to affect the observable shape, particularly of organisms which have semiplastic bodies. Some organisms can produce stems, and some of these stemmed forms produce colonies. Colonies may be flat, spherical, linear or have treelike variations.

### 4. *Internal Structures*

a. *Contractile vacuoles.* The contractile vacuole is the pump which removes excess water and soluble wastes from the protozoan body. In flagellates the contractile vacuoles are always spherical. Contractile vacuoles are not found in some flagellates but most flagellates have one or more, the number often being characteristic of a species. They are usually located anteriorly but other arrangements may be seen and their position is usually constant in a given species. In small colorless flagellates the vacuoles are so near in appearance to the cytoplasm that they are often very difficult to find. No vacuole should be considered to be contractile unless seen to discharge or known to disappear and reappear.

b. *Food vacuoles.* These are usually similar in appearance to contractile vacuoles but occasionally may be irregular in shape. They are found at any location within the cell and do not discharge periodically as do the contractile vacuoles, but move about in the cell. Their presence indicates that the organism consumes particulate food, such as bacteria, other protozoa or algae, which may be seen inside them. Occasionally particulate food is ingested without a definite food vacuole being observable.

c. *Stored food.* Starch, glycogen (animal starch), paramylum (a starchlike carbohydrate), leucosin (probably a polysaccharide carbohydrate) and volutin (protein) are stored by various types of cells as solid food reserves. Some species also store fatty materials in the form of oil droplets. Starch is characteristic in appearance and responds to the iodine test, yielding a blue to purple color. Glycogen yields a reddish color with iodine. Paramylum and leucosin are not colored by iodine but they are characterized respectively by disc, ring, brick or rod shapes for paramylum, and glistening spheres for leucosin. Volutin is deposited as angular bodies and takes up stains used in staining certain cell constituents.

d. *Chromatophores* (Figs. 5b and 6). These are colored bodies and include the chloroplasts by which the green, yellow-green, brown and blue-green forms manufacture food materials such as starch and paramylum, using sunlight as "fuel." These colored plastids or chromatophores (also called chromoplasts) number from one to approximately fifty depending on the species and may be disc, cup, bowl, trough, star or otherwise shaped. The presence or absence of chlorophyll-containing chromatophores (called chloroplasts if distinctly green in color) divides the flagellates into two distinct groups: the green (also brown or otherwise pigmented) or plant flagellates, and the colorless or animal flagellates. A number of colorless flagellates, however, have such evident close relationships to forms having chloroplasts or chromatophores that they are classified with the plant flagellates.

e. *Pyrenoids.* These structures are mentioned here not because they are easily distinquishable, for they are difficult to observe, but because they are important for identifying certain organisms and are believed to have an important function. They are very small, dense, colorless bodies associated with the chloroplasts (Fig. 5). They may be located deeply within, or partly exposed at the surface of the chloroplasts. They are frequently covered with a sheath of carbohydrate—usually starch. These bodies are believed to be the centers of carbohydrate production, but some organisms which produce starch have no discernible pyrenoids. Starch is also produced by some organisms which are colorless. Starch sheaths may be more readily found by treating with iodine solution (see Appendix A).

f. *Stigma.* These bodies are also known as "eyespots." They are almost always red and, if present, are usually located near the base of the flagella. In a few organisms they are in the posterior half. They may be spherical, discoid, bar or rod-shaped. They are often compact and dense, but in some organisms they may be a more or less loose collection of granules or droplets. The stigmas (Figs. 5b and 6) often appear as dark spots with usual microscopic lighting but, if the diaphragm is opened wide to obtain the greatest amount of light, they will ordinarily show their bright red color. Occasionally they are orange-red and infrequently almost purple. In most flagellates possessing a stigma, it is believed their response to light is controlled by the stigma. Stigmas are much more common in green forms than in colorless ones.

g. *Nucleus.* The nucleus might be called the life center of the cell since it has been shown that a cell without a nucleus dies. The typically spherical nucleus of the flagellates is usually somewhat centrally placed in the cell but in some groups it is displaced (anteriorly in *Mastigamoeba*) as has already been indicated. The vesicular nucleus is almost universal among the flagellates. It consists of a dense central body, the endosome, surrounded by clear nuclear sap which, in turn, is enclosed by the nuclear membrane. Granules of chromatin are suspended in the nuclear sap. Strands suspending the endosome have been demonstrated in some nuclei. The nuclear membrane is sometimes lined with scattered granules. The endosome may be lacking in some vesicular nuclei. The nucleus is best studied by means of nuclear stains of which iron-hematoxylin is much to be preferred, but discussion of such preparations is beyond the scope of this book. The methyl green-acetic acid mentioned in Part I is readily used and is serviceable for demonstrating the location of the nucleus. Methyl green stain often yields a pale green nucleus which is easily overlooked. The parabasal body or kinetonucleus is also stained by methyl green and should not be confused with the nucleus. It is solid and thus lacks the structure of the vesicular nucleus. Organisms with kinetonuclei also have true nuclei which can usually be found with a little searching.

### 5. *Motion*

The manner in which most individual species of flagellates move is frequently characteristic for that species or for the genus to which it belongs. In general, unattached forms either swim freely in the liquid or move across the substrate. The swimming motion of the free forms is usually a spiral (helical) progression. Swimming may be practically continuous, noticeably intermittent, or sometimes jumpy. It is frequently in a linear direction circumscribed by the helix, but meandering and circling paths characterize certain organisms. Forms that move over a substrate show even more distinct patterns of progress than do the free swimming ones. The particular type for an organism is dependent (1) on the action of the swimming flagellum or flagella, (2) upon whether part or all of the body contacts and drags on the substrate, and (3) upon the movement of the body itself (separate from the effect of the flagellum). These work separately or together to produce jumping, quivering, vibrating, swaying, contorting or some other type of motion. In many cases it is characteristic enough for species to be recognized with fair assurance. The flagella usually play an important part; they may vibrate in part or whole, hook, strike backwards, act in a wave-like manner or other descriptions might apply. They are usually carried forward but in a few organisms operate obliquely to the body axis and thus produce a characteristic movement. If the organism is pleomorphic (having more than one form) the motion or the flagellar action can be of distinct help in recognizing the less usual forms. Some organisms are, at times at least, amoeboid. Even attached forms often show characteristic actions (see *Pleuromonas jaculans*).

## CLASSIFICATION (OR TAXONOMY)

In any discussion of heterogeneous objects, it is usual to group them into divisions in which all the objects in one division are somewhat related to each other. These divisions may then be subdivided into smaller and more closely related groups. The same methods are applied to protozoa. In the first grouping they are divided into two classes: the plantlike flagellates, Phytomastigophorea, and the animal-like flagellates, Zoomastigophorea. The scientific names tend to make persons with little scientific background shy away from further discussion of the subject. They ask immediately, "Why couldn't they be called the plant flagella bearers?" (That is the actual meaning of Phytomastigophorea). However, we are indebted to German and French workers for much of our present knowledge concerning these forms. If the names had been given in German and French, we would no doubt have English translations of some of them and others left in the original language, which would make the problem even more difficult than it now is. Fortunately, scientists have agreed to use Latin (or Greek) names for organisms. When the naming of many flagellates occurred, Latin was a more universal language than it now is, but even today Latin is a satisfactory universal scientific language. As may be seen from the above, a name has meaning, and the ending on the name indicates which step in subdividing has been reached. Each subdivision is composed of one or more lower groups.

In classifying *Peranema trichophorum*, one of the very common flagellates of sewage treatment, the following are the divisions and subdivisions used:

Subphylum Mastigophora
**CLASS PHYTOMASTIGOPHOREA**
**Order Euglenida**
**Suborder Peranemoidina**
Family Peranemidae
Genus *Peranema*
Species *trichophorum*

It may be seen that the name of the organism is its generic name *Peranema* plus its specific name *trichophorum*. The name of the person who originally described the organism is frequently written after it to definitely identify the organism—thus, *Peranema trichophorum* Ehrenberg. It is also frequently found written *Peranema trichophorum* (Ehrenberg) Stein, which indicates that Stein has changed the name originally given by Ehrenberg.

The classification used in this book is, in general, the classification proposed by Jahn and Jahn (7) and modified by Hall (4). Certain minor changes have been introduced, and in some groups the classification of Kudo (8) has been followed because it seemed more logical.

Before discussing the entire group of flagellates which have been reported from sewage treatment, it seems desirable to discuss several that are most frequently encountered and by which the person with little experience may orient himself. These organisms are described more completely elsewhere in this book.

*Peranema trichophorum:* This is the largest flagellate commonly encountered in sewage treatment. Its anterior flagellum is heavy and carried stiffly forward with the anterior third or fourth being vibratile. It possesses a short trailing flagellum which is usually not seen. The organism moves steadily but slowly and with considerable contortion of the body (metaboly). It stops, folds on itself and moves off in a different direction. It glides along the substrate but only rarely swims freely. Its usual size is about $50\mu$. It is found most frequently in trickling filters but it is also present in sand filters, in Imhoff tanks, sometimes in activated sludge and frequently in other locations. It is not confined to sewage and may be found in relatively fewer numbers in unpolluted waters (see Fig. 76).

*Bodo caudatus*: This small flagellate possesses both swimming and trailing flagella. The swimming flagellum has a hooking action which carries the organism over the substrate as it lies on one side. It very rarely swims freely in the liquid at a distance from the substrate. The shape shown in Figs 1 and 113a, c is usually seen, but Figs. 113d, e, f, g indicate variations which may occur. This organism is found in starting activated sludge, in Imhoff tanks, and in raw sewage and will develop in trickling filter sludge that is held for a time. Strongly aerobic or strongly anaerobic conditions seem to kill it. The usual size is 17μ.

*Oicomonas* spp: There are several kinds of these (see Figs. 13 through 17) that occur frequently, although usually only one or two species are present at the same time. The eccentric origin of the flagellum, the liplike projection and the stiffiy curved manner in which the primary flagellum is held are typical of all. The flagellum sometimes shows an undulating or wavy type of motion. All *Oicomonas* species possess the ability to form posterior protoplasmic stems, and at least one forms colonies. The environmental requirements appear to be similar to those for *Bodo caudatus*. They are especially numerous in starting activated sludge. Size varies with species but all those commonly found in sewage are under 20μ; some are very small (5-9μ).

*Trepomonas agilis*: This posterio-laterally winged flagellate (see Fig. 146) of the so-called two-mouthed group possesses flagella in two laterally placed groups. Only one flagellum on each side can be distinguished with ease. Each flagellar stroke turns the organism about ¼ turn. The end view is confusing until a lateral view is obtained since the end view is either "S-" or comma-shaped. This is the most common flagellate in raw sewage, primary tanks, Imhoff tanks and similar environments. It is numerous in anaerobic environments but is not ordinarily found in separate sludge digesters. Size is variable but it is usually 20μ or smaller, more commonly 8-10μ.

*Hexamitus* spp.: There are several species in this genus, all of which have eight flagella. Two of the flagella are trailing and three swimming flagella arise on each side near the anterior end. *Hexamitus* is often found associated with *Trepomonas agilis* in materials that are becoming anaerobic. Under such circumstances, rotifers and other metazoa will be killed and *Hexamitus* will invade these dead organisms. Most of these are under 25μ long (see Figs. 141-145).

The novice should be able to find one to several of the above protozoa. Examination of them will yield understanding of some of the terms used in describing organisms.

## EXPLANATION OF DESCRIPTIONS AND ILLUSTRATIONS

Pascher's *Die Susswasserflora Deutschlands, Osterreichs und der Schweiz* (12, 13, 14) is a good reference in identifying the organisms discussed here. Wherever possible, individual species have been studied and the descriptions verified. Drawings have been revised or redrawn when it was evident from study that existing illustrations left something to be desired. The drawings were made at the magnification most likely to be used in viewing the organism, and illustrations copied from other sources have been brought to the same magnification (usually 430X). This gives the comparative size of the organism at a glance. Where the illustration at this power was too small to show detail, it has been shown on an enlarged view.

In defining classes, orders, and families, those organisms chiefly associated with sewage and waste treatment have been considered. For this reason, the characteristics of a group as given here may differ in some slight detail from descriptions found elsewhere. Usually the description applied to protozoa of sewage and waste treatment does not need to be so broad as that required for a more inclusive discussion.

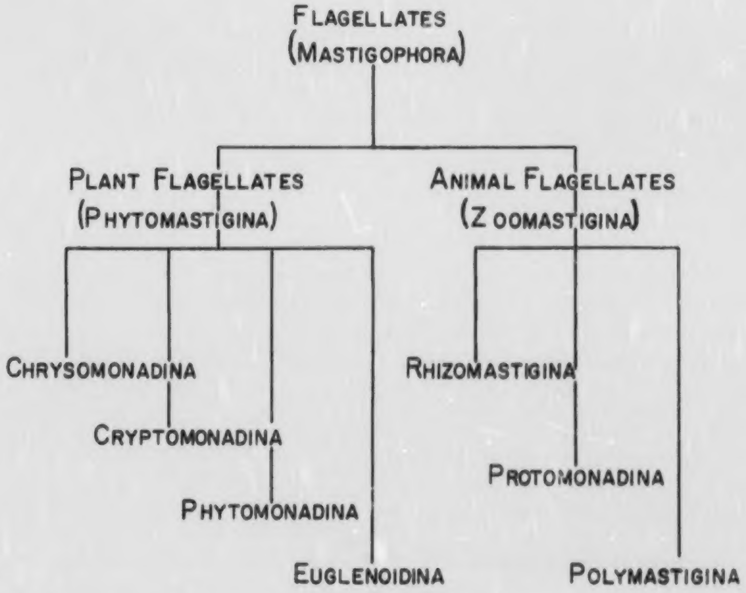
FLAGELLATES
(MASTIGOPHORA)
PLANT FLAGELLATES
(PHYTOMASTIGINA)
ANIMAL FLAGELLATES
(ZOOMASTIGINA)
CHRYSOMONADINA
CRYPTOMONADINA
PHYTOMONADINA
EUGLENOIDINA
RHIZOMASTIGINA
PROTOMONADINA
POLYMASTIGINA

Fig. 7 Phylogenetic family tree of the flagellates

# PART III

## CLASSIFICATION AND DESCRIPTION

Subphylum Mastigophora (Flagellates): Organisms which are motile by means of flagella (except as noted below) or which, if attached, draw food to themselves by means of flagella. Longitudinal division is typical ( other types will be noted if present). Contains two classes:

*CLASS PHYTOMASTIGOPHOREA*: The plantlike flagellates. These flagellates possess chromatophores or have characteristics similar to those of other organisms which possess chromatophores. Contains seven orders, of which four are encountered in waste treatment and polluted streams (see Fig. 7).

*CLASS ZOOMASTIGOPHOREA*: Animal flagellates. Flagellates without chromatophores and not showing close affinities to organisms possessing chromatophores. Contains five orders, of which three are encountered in waste treatment (Fig. 7 and p. 86).

### CLASS PHYTOMASTIGOPHOREA

**Order Chrysomonadida:** Minute yellow, greenish-yellow, greenish-brown, or colorless flagellates with one or two flagella. Chromatophores, if present, one, two or rarely more. Some are armored or live in shells. Some normally form colonies. While no record has been found of the occurrence of colored forms in sewage treatment processes, they do occur and multiply in streams which have received sewage. The following colored forms have been reported from such streams.

FAMILY CHROMULINIDAE: Chrysomonadida with one flagellum.

*Genus Chromulina*: Small naked crysomonads with one bandlike chromatophore.

*Ch. ovalis Klebs*: (Fig. 8 a,b,c) Size, 9 to 14$\mu$ by 6 to 7$\mu$. Only the genus is ordinarily reported but this is the most probable species. A flexible and almost amoeboid chrysomonad which generally assumes a nearly ellipsoidal shape. The anterior end is almost invariably indented. Other variations of cell shape frequently encountered are oval to heart-shaped with a bluntly pointed posterior end. Changes in shape are quite marked, although the organism seldom pro-

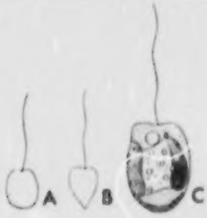

Fig. 8 *Chromulina ovalis*
a. Outline
b. Outline of another form
c. Detail

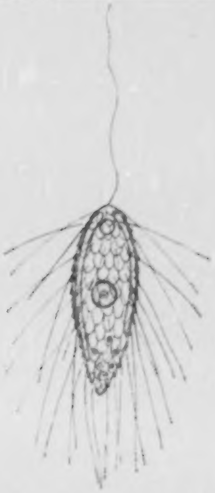

Fig. 9 *Mallomonas caudata*

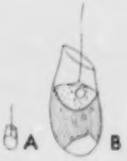

Fig. 10 *Kephyrion ovum*
a. Outline
b. Detail

duces distinct pseudopodia. Optical cross section circular. Flagellum 1½ times body length, practically apical in position. Chromatophore, a disc or a band, yellow to brown in color which is of variable density. Holophytic, but sometimes ingests protozoa and algae. These foods are probably taken in by means of posterior amoeboid processes. The small rounded eyespot is near the flagellum base as are also two contractile vacuoles. Encystment occurs and a palmelloid state exists. Pellicle unmarked, nucleus indistinct. Probably not found where pollution has been recent.

*Genus Mallomonas*: These are usually relatively large monads (monads are small mono- and bi-flagellated organisms) having a covering of overlapping siliceous plates. These plates may be in rows or irregularly arranged. Siliceous needles arise from a jointed attachment to the plate. The needles are usually long and delicate. They may be hollow and sometimes have toothed ends. In some species they are on one end of the cell, in others on the other end, on both or sometimes covering the entire cell. There are two large bowl-shaped chromatophores oppositely placed. Contractile vacuoles, four or more, and variously placed. The organisms frequently possess an anterior noncontractile vacuole. The single thick flagellum is retractible through a flagellar slit. Stored food: large clumps of leucosin and small droplets of oil. These organisms cause taste and odor in water supplies.

*M. caudata* Iwanoff: (Fig. 9) Size, 50 to 80$\mu$ by 15 to 30$\mu$. Only the genus has been mentioned in any literature covered by this work. Liebmann (11) mentions this species in his discussion of stream biota, but takes the stand (as does Pascher (13)) that it is found only in clean waters. Inverted pyriform* with the posterior end drawn out like a tail. Chromatophores, two, large, oppositely placed and just below the pellicle layer. Scales oval, imbricated, without definite lines of arrangement. Needles body length, more or less outwardly pointing and toothed along one side, frequently forked. Cysts 20-30$\mu$ diameter. This is a widely distributed planktonic form.

*Genus Kephyrion*: These minute, loricated flagellates ordinarily occupy only ½ to ⅔ of the bottom of the lorica. This shell is spindleform or pyriform, slightly more narrow at the anterior end. The single chromatophore is ring-form and equatorially placed. A single flagellum is apically located.

*K. ovum* Pascher: (Fig. 10 a,b) Size of shell, 7$\mu$ by 4$\mu$. This flagellate is obliquely truncated anteriorly and the actual protoplast may be wider than long. It occupies only the bottom of the lorica. The lorica is also obliquely truncated. The single ring-form chromatophore lies close to the lateral walls. This yellow-brown chromatophore and the brown shell give the entire organism a brown coloration. Flagellar length approximately length of shell.

FAMILY SYNCRYPTIDAE: Chrysomonads with two equal flagella.

*Genus Synura*: Colonial forms in which the posterior of the cell is drawn out and the ends of the cells united, forming elongate, oval or spherical colonies. The cells lie close to one another. The thick pellicle has bristles, warts or lines. Chromatophores are brown, two, oppositely placed, arched. No stigma. Several contractile vacuoles which are perhaps united into a system. A much enlarged vesicle is often found anteriorly. The two equal flagella are 1½ times length of cells.

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*The term pyriform is used to indicate "candle flame shape" and if unmodified usually indicates that the flagella spring from the more pointed or "top" end. Inverted pyriform indicates the flagella emerge from the broad and more rounded end.

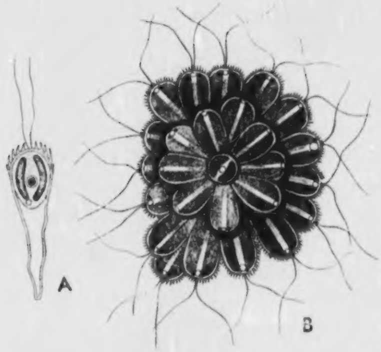


Fig. 11 *Synura uvella*
a. Optical cross section of single cell, showing detail
b. Colony

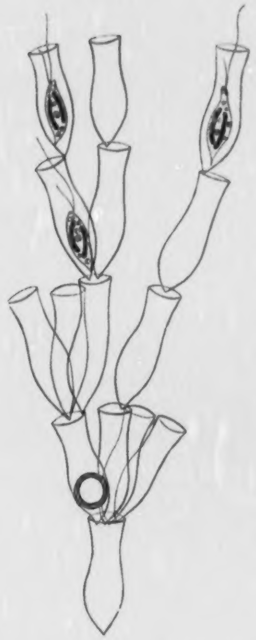


Fig. 12 *Dinobryon sertularia*—colony with detail of some single cells

*S. uvella* Ehrenberg: (Figs. 11a,b) Size of cells, 20 to 40μ by 8 to 17μ. Cells mostly inverted pyriform. Bristles very distinct, often long. Colonies 100 to 400μ diameter.

FAMILY OCHROMONADIDAE: Organisms with two unequal apical or slightly lateral flagella.

*Genus Dinobryon*: This chrysomonad forms arboroid colonies by building vaselike loricae which are attached, one above another, each just within the opening of the mother lorica. Both flagella are active and usually, but not always, inserted on a forward projection of the protoplast. The protoplast is spindleform to nearly cylindrical and is attached near the bottom of the lorica by a contractile protoplasmic thread upon which it may extend itself to the flared opening of the lorica. The brownish-yellow chromatophores are large, usually two, oppositely placed and frequently unequal in size. Anterior contractile vacuole variable. Eyespot usually visible, anteriorly placed. Stored food reserve, leucosin, as glistening bodies, usually in the posterior of the protoplast but may be scattered throughout the body.

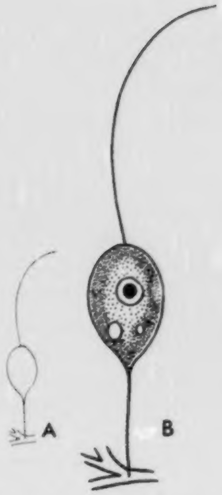
*D. sertularia* Ehrenberg: (Fig. 12) The pointed vaselike individual loricae produce a colony, the branches of which do not diverge widely. The yellow color and appearance of the colony remind one of a head of oats. Individual loricae are 30 to 44$\mu$ by 10 to 14$\mu$. The protoplast is usually spindle-shaped with a round or obliquely truncated anterior face.

The secondary flagellum usually reaches just beyond the opening of the lorica. The primary flagellum is about as long as the lorica. A single contractile vacuole occurs near the base of the flagella. Leucosin, as spherical granules or discs of various sizes, may be scattered throughout the cytoplasm but is usually more concentrated in the posterior portion. Two, occasionally one, brownish-yellow chromatophores, curved, lying in an approximately anterior-posterior direction. If two, they are frequently unequal and opposite each other but not closely adjacent to the lateral walls. The entire colony swims but the swimming action is rather feeble. The central vesicular nucleus is practically indistinguishable. The almost transparent loricae are homogeneous and are reported to be composed of cellulose and silica. They become yellow to brown in color due to iron impregnation. Faint rings, or annulae, may sometimes be seen in the neck region. The loricae stain with gentian violet.

The following colorless forms are found frequently in sewage treatment processes. All belong to the colorless Ochromonadidae:

*Genus Oicomonas*: This genus was classified by Pascher (12) under Protomastiginae (equivalent name—Protomastigida). Hall (1953) (4) classifies *Oicomonas* as a colorless chromulinid following the usual description that *Oicomonas* has only one flagellum. Examination of members of the genus reveals several species having a short secondary flagellum. Pascher himself observed one such and other authors agree that it is a common occurrence. It would, therefore, appear that *Oicomonas,* if it is to be classified as a colorless chrysomonad, does not belong to the Chromulinidae which is reserved for chrysomonads with one flagellum, but rather belongs to the Ochromonididae which are chrysomonads having two unequal flagella.

Oicomonads show strong amoeboid tendencies and almost all produce posterior protoplasmic stems. A liplike extension on one side of the anterior end is characteristic but is



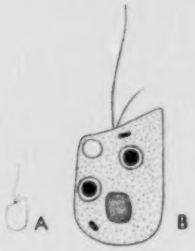


Fig. 14 *Oicomonas ocellata*
a. Outline
b. Detail

Fig. 13 *Oicomonas mutabilis*
a. Outline
b. Detail

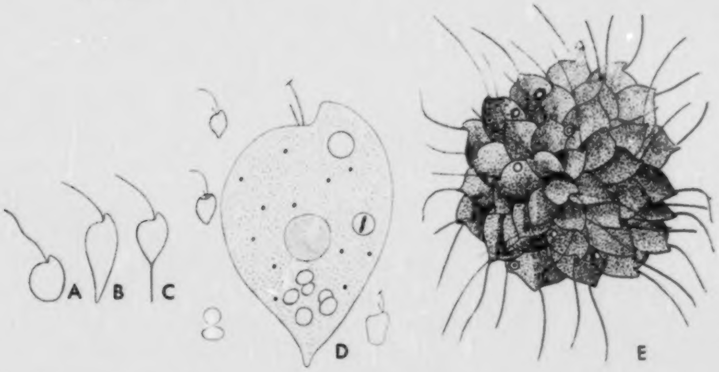


Fig. 15. *Oicomonas socialis*

a.
b. } Outlines showing possible variations
c.

d. Detail, showing vacuole containing bacteria and showing variations possible in the free-swimming cells, and cross sections (lower left)—small figures not to size.
e. Colony

variable in size. The primary flagellum sometimes exhibits a wavelike action but is usually carried stiffly in a curve high over the anterior end, vibrating while thus held. The secondary flagellum, if present, lies in the angle between the primary flagellum and the anterior face. It vibrates actively and may at times be the sole producer of the current bringing food to the body. Both flagella are laterally displaced, and only rarely will they appear apical if the organism is viewed from several angles. Food is taken in near the base of the flagella by thrusting upward a cuplike pocket which quickly encloses the food particle. The pocket, upon closing, moves laterally and posteriorly forming a large food vacuole which persists as a distinct outward bulge until the posterior portion of the organism is reached. At this point it seems to penetrate inward and becomes less evident. These organisms are voracious feeders on bacteria.

The free swimming individuals of the various species of this genus are quite different in appearance from the attached individuals.

*O. mutabilis* S. Kent: (Fig. 13 a,b) Size, 16 to 17$\mu$. Cells almost spherical, oval, or inverted pyriform; free swimming or attached; posterior often drawn out into a long stem. Primary flagellum almost double the body length. Nucleus somewhat forward of the middle. Two contractile vacuoles in the posterior end. Found where organic decomposition is active.

*O. ocellata* Scherffel: (Fig. 14 a,b) Size, 8 to 12$\mu$ by 8$\mu$. Cells oval or rounded; anterior drawn out liplike, with a ruby red to almost black eyespot. Primary flagellum somewhat over body length. Nucleus almost central. One anterior contractile vacuole.

This species, or a similar one, frequently forms colonies in starting activated sludge. The colonies are generally compact reminding one of *Synura* colonies and swim with a rolling action.

*O. socialis* Moroff: (Fig. 15 a,b,c,d,e) Size 10 to 15$\mu$ by 10 to 12$\mu$, pyriform, anterior end with a sharply pointed lip and with a posterior joined to others to form a free swimming colony of similar cells. Primary flagellum may be double the body length; nucleus posterior of middle. One contractile vacuole lateral in midsection. Found where

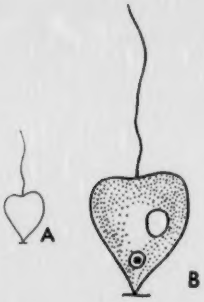
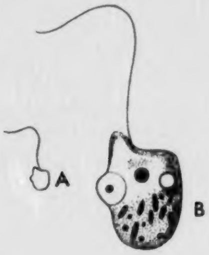


**Fig. 16** *Oicomonas steinii*
a. Outline
b. Detail

**Fig. 17** *Oicomonas termo*
a. Outline
b. Detail—bulge on left side is vacuole containing a recently ingested bacterium.



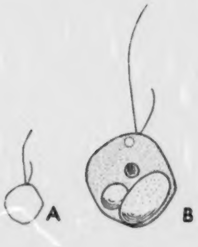
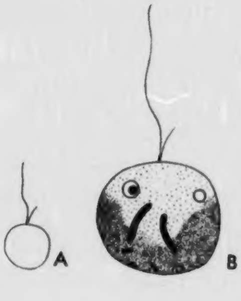


**Fig. 18** *Monas amoebina*
a. Outline
b. Detail

**Fig. 19** *Monas arhabdomonas*
a. Outline
b. Detail



decomposition of organic matter is active. It is reported (9) as commonly found in Imhoff tanks. Colonies tend to be looser in construction than those mentioned above (see *O. ocellata*). Dissociation of colonies into small groups of individuals commonly occurs.

*O. steinii* S. Kent: (Fig. 16 a,b) Size, 16 to 17μ. Cells of variable form; when attached, broad pyriform with a rounded anterior border. Primary flagellum about body length, nucleus posterior.

*O. termo* (Ehrenberg) S. Kent: (Fig. 17 a,b) Size, 5 to 9μ. Cells oval to spherical, anterior drawn out liplike. Flagellum about double body length. Nucleus and one contractile vacuole in anterior end. Poly-and mesosaprobic.

*Genus Monas*: Cells generally spherical to ovoid with delicate cuticular layer, changeable in form, free swimming or attached by a delicate thread which extends from the posterior end, mostly single, seldom united into an actual colony. Flagella (one long and one, or possibly two, short) set in a rounded anterior end, normally each with basal granule and rhizoplast. Anterior end sometimes possessing a slit or pocketlike depression. Eyespot present or absent. One contractile vacuole. Oil droplets present in cytoplasm, frequently leucosin also. Encystment known.

This genus was classified under the Protomastiginae by Pascher (12) but Hall (4) includes it in the Family Ochromonadidae. The difference between *Monas* and *Oicomonas* is not altogether clear.

*M. amoebina* H. Meyer: (Fig. 18 a,b) Size, 12 to 15μ. Cells spherical, strongly changeable in form, free swimming. Ingestive area present, eyespot absent. Posterior end containing leucosin. Primary flagellum 1½ times body length. One contractile vacuole in the anterior end. Cysts not known. Nutrition holozoic to saprophytic.

*M. arhabdomonas* (Fisch) H. Meyer: (Fig. 19 a,b) Size approximately 17μ. Cells spherical to broadly pyriform. Free swimming. Primary flagellum slightly over body length. Mouth area and eyespot absent. One contractile vacuole adjacent to nucleus in anterior end. Cysts known. Nutrition holozoic. Mesosaprobic.

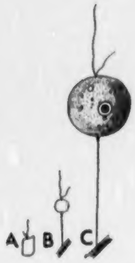


Fig. 20 *Monas nimima*
a. Outline of free-swimming cell
b. Outline of attached cell
c. Detail of attached cell

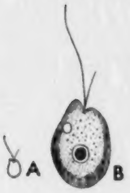


Fig. 21 *Monas obliqua*
a. Outline
b. Detail

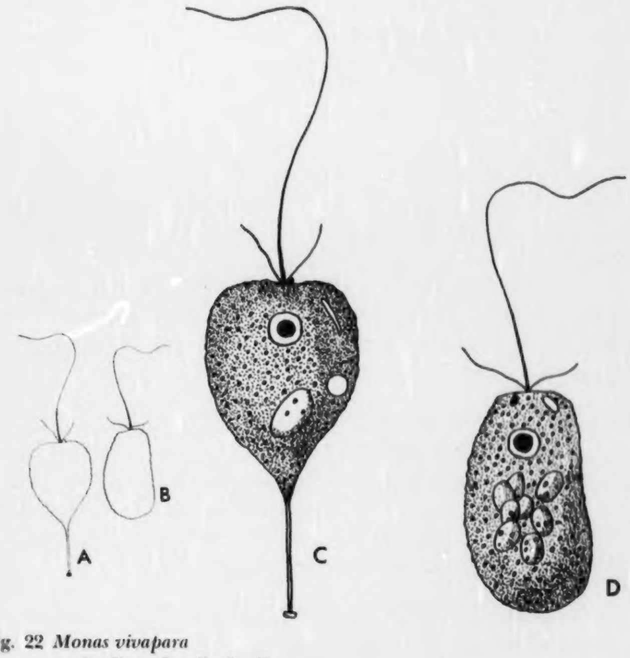


Fig. 22 *Monas vivapara*
a. Outline of stalked cell
b. Outline of free-swimming cell
c. Detail of stalked cell
d. Detail of free-swimming cell

*M. minima* H. Meyer: (Fig. 20 a,b,c) Size, 4 to 7μ by 3 to 5μ. Cells spherical, oval or almost cylindrical. Free swimming or attached. Mouth area and eyespot absent. Primary flagellum body length. One anterior contractile vacuole. Cysts not known (1914). Nutrition holozoic and saprophytic. Mesosaprobic.

*M. obliqua* Schewiakoff: (Fig. 21 a,b) Size, 6μ. Cells spherical to oval, anterior end oblique. Free swimming. Mouth area and eyespot absent. Primary flagellum somewhat more than body length. One antero-lateral contractile vacuole. Cysts not known (1914). May be identical with *Oicomonas termo.*

*M. vivipara* Ehrenberg: (Fig. 22 a,b,c,d,) Size, 20 to 40μ. Cells spherical, elongated or pyriform. Free swimming or attached. Primary flagellum approximately 1½ times body length. Mouth area and eyespot present. Contractile vacuole lateral in midsection. Cysts known. Nutrition holozoic. Mesosaprobic.

*M. vulgaris* (Cienk) Senn: (Fig. 23 a,b) Size, 14 to 16μ. Cells spherical, pyriform or oval. Free swimming or attached. Primary flagellum about 1 to 2 times body length. Mouth area present. Eyespot absent. Contractile vacuole anterior adjacent to the nucleus. Cysts formed. Nutrition holozoic. Mesosaprobic.

*Genus Physomonas*: Cells stalked at times and covered with a finely granular siliceous layer having radiating threads. Mouth area present. Two contractile vacuoles. Reproduction and cysts not known (1914) (12). Nutrition holozoic by formation

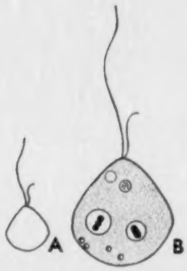


**Fig. 23 *Monas vulgaris***
**a. Outline**
**b. Detail**

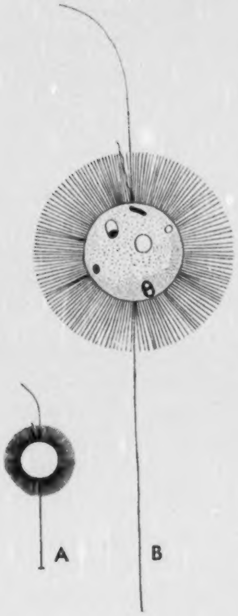


**Fig. 24** *Physomonas vestita*
a. Outline
b. Detail

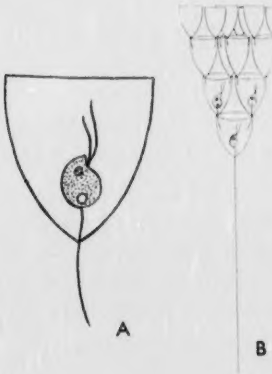


**Fig. 25** *Stylobryon abbottii*
a. Single Cell
b. Colony

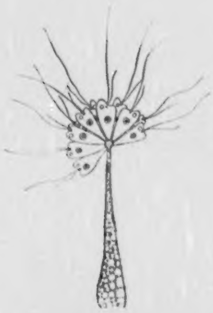


**Fig. 26** *Anthophysa vegetans*
a. Colony

of cup and food vacuole somewhat after the manner of the oicomonads but vacuole enters more directly into body.

*P. vestita* Stokes: (Fig. 24 a,b) Size 12 to 15$\mu$. Cells spherical. Stem about 3 times body length. Primary flagellum two times body length, secondary flagellum body length. Contractile vacuoles in anterior half. Has been classified by some authors under *Monas,* and Pascher (12) suggested that it might be transferred to the chrysomonads.

*Genus Stylobryon*: Cells with delicate pellicle layer, possessing an anterior lip. Fastened by means of delicate thread to the base of the lorica. The lorica is attached by means of a short stem to the inside of the next oldest lorica, thus building a branched treelike colony. One contractile vacuole. Cysts not known (1913) (12). Nutrition holozoic.

*St. abbottii* Stokes: (Fig. 25 a,b) Size, 17$\mu$. Lorica wide cone-shaped with convex walls, hyaline to chestnut brown, approximately twice as long as wide. Stem from bottom of first lorica approximately 100$\mu$ long. Other stems about 8.5$\mu$. Cells oval or almost spherical. Protoplast does not fill width of lorica. Primary flagellum about body length, secondary flagellum slightly shorter. Contractile vacuole in posterior end.

*Genus Anthophysa*: Cells with delicate pellicle. An extended anterior liplike structure. Posterior end drawn out pseudopodlike. One species with eyespot. Forms clusters of many cells on the end of yellow or brown, branched, contorted stems. Colony disintegrates and the individuals become free swimming when exposed to strong light. Flagellum with a basal granule which is attached by means of a rhizoplast to the nucleus. One contractile vacuole. Conjugation reported with zygote formation. Nutrition holozoic.

*A. vegetans* (O. F. Muller) Stein: (Fig. 26) Size, cells 3 to 10$\mu$. Cells inverted pyriform or wedge-shaped, united into a spherical colony on the end of a stem. Eyespot absent. Contractile vacuole anterior. Primary flagellum 1½ to 2 times body length, secondary flagellum ⅛ body length. According to Pringsheim* this organism may be synonymous with *Oicomonas socialis* and *Monas sociabilis.*

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*Verbal communication.

**Order Cryptomonadida:** Body more rigid and more differentiated than the previous order. Typically with a flattened ventral side, an arched dorsal side and an oblique furrow indenting the anterior end. This furrow often sinks deep into the body as it crosses the anterior face forming a V-shaped, bowl-shaped, or cylindrical gullet, or it may be open ventrally forming a groove. Flagella two, unequal to almost equal, originating from the gullet or from the anterior median portion of the furrow. The gullet and groove are often lined with refractile granules, apparently trichocysts, usually visible in living specimens. One to several chromatophores. The chromatophore is closely adjacent to the pellicle and is usually olive green, less commonly green, brown, blue-green or red. Metabolic products, starch and oil. Nucleus usually posterior. Contractile vacuoles 1 to 3 in the anterior end, opening into the furrow. Palmella state exists for many species. Pascher states, "often associated with euglenoids and particularly common in waters rich in organic matter and also in waste waters, compost drippings . . . ." In spite of Pascher's views, the review of the literature revealed no case in which chromatophore-bearing forms have been reported from waste treatment processes.

FAMILY CRYPTOCHRYSIDAE: Cryptomonads of rather simple organization. Furrow not deepened into a gullet in the protoplasm but may appear as a groove on the ventral side. Groove and perhaps part of furrow bordered with refractile granules. Flagella apical or subapical in position with flagella insertions shifted ventrally in the latter group.

*Genus Chroomonas*: Cells long pyriform to ellipsoid; posterior rounded, anterior obliquely truncated, distinctly dorso-ventral. Chromatophores single, or two, adjacent to the lateral walls, bowl-shaped and frequently definitely overlapped; blue or blue-green in color. Pyrenoid develops two starch covers. Furrow weak, often obliterated. Flagella two. Nucleus posterior (without exception?). Contractile vacuole in anterior end.

*C. setoniensis* Lackey: (Fig. 27 a,b) Size, about 15$\mu$ by 15$\mu$ by 8$\mu$ thick. Cell constant in shape, flattened, oval, slightly obliquely truncated at the anterior end, with a shallow gullet or mouth depression, open along one side. Two subequal flagella emerge from the mouth depression, the longer about 25$\mu$, the shorter about 20$\mu$. The single chromatophore is bright blue having the form of a peripheral cylinder, split from anterior to posterior with irregular

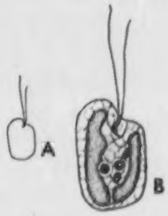


Fig. 27 *Chroomonas setoniensis*
a. Outline
b. Detail

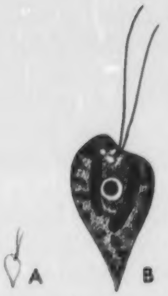


Fig. 28 *Chroomonas cyaneus*
a. Outline
b. Detail

edges. One simple contractile vacuole near the point of emergence of the flagella. The nucleus is median with a thin membrane and no peripheral chromatin granules. Stained preparations show that the flagella end in small basal bodies with no visible rhizoplast. There are several types of inclusions among which are spheres, which might be oil, and bodies resembling paramylum. This species swims rapidly, revolving on its main axis. No indication of the means of reproduction has been seen.

*C. cyaneus* Lackey: (Fig. 28 a,b) Size, never exceeding 10$\mu$. Organism inverted pyriform, obliquely truncated anteriorly, tapering to a somewhat extended point posteriorly. Two equal or subequal flagella which emerge from a slight depression anteriorly; they are about body length, directed forward in swimming. Two or more contractile vacuoles are located at their base. There is no eyespot nor can other structures be distinguished in this region. The nucleus is central and apparently there is no endosome, only a granular mass of chromatin. Chromatophores, two in number, band-shaped, curved, of bright blue color which speedily diffuses into surrounding water when the organism disintegrates. Pellicle thin, rigid. Swimming path a helix. Not found in sewage but found in a heavily polluted stream.

*Genus Chilomonas*: Cells colorless, laterally compressed. When viewed from the broad side, the anterior end appears laterally, obliquely and irregularly truncated. It is somewhat flattened ventrally and strongly arched dorsally and has the anterior dorsal portion drawn out into a lip or edge. Posterior round or sometimes narrow and stumplike. In the anterior border there is a short, irregularly oriented, furrow which invaginates almost to the middle of the organism or beyond as a smooth, irregular open pharynx and ends near this point as a blind sack. The pharynx is lined with almost spherical granules or trichocysts. The pellicular layer is distinct and in some species dense, having longitudinal fibrillae. The subequal flagella are shorter than the body and inserted somewhat ventrally. Nucleus relatively large and posterior to the cell middle. One or two contractile vacuoles in the anterior, dorsal end. Cells often filled with polygonal starch granules which lie close to the walls and obscure the cell details. Cysts known. May be found in very polluted conditions.

*Ch. paramecium* Ehrenberg: (Fig. 29 a,b) Size, $20\mu$ to $40\mu$. Oblong cylindrical shape; posterior end of body with a tail-like narrowing which is often bent dorsally. Saprophytic.

FAMILY CRYPTOMONADIDAE: The pharynx is not open ventrally over most of its length. More complex in organization than the previous family. Pellicle layer distinct, trichocyst layer of pharynx usually easily distinguishable.

Dorso-ventral nature frequently quite apparent and the apical furrow may be distinguished in most species after a little study.

*Genus Cryptomonas*: Cells with definite dorsal and ventral sides. Usually with flat or even concave ventral side and often with a highly rounded dorsal side. Anterior end is laterally truncated by means of an almost apical furrow. The anterior usually has an extended border. The opening of the pharynx is located here. Two flagella are inserted in the furrow or the mouth of the pharynx. Nucleus usually posterior. Chromatophore, one or two closely adjacent to the lateral walls, often overlapped and divided into several smaller portions. Metabolic products, starch or starchlike (producing red to blue color with iodine) in the form of small discs. Pellicle layer distinct, often weakly striated. Trichocysts on ventral side of pharynx and furrow region. One to three contractile vacuoles in the anterior end and emptying into the furrow. Palmella state known; division usually takes place in this state.

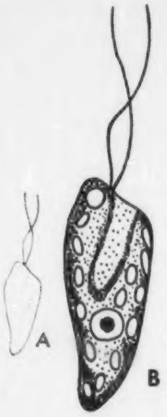


Fig. 29 *Chilomonas paramecium*
a. Outline
b. Detail

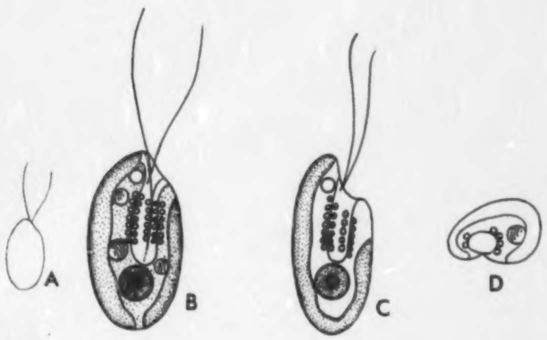


Fig. 30 *Cryptomonas erosa*
a. Outline
b. Detail from dorsal side
c. Detail from lateral side
d. Cross section through gullet area

*C. erosa* Ehrenberg: (Fig. 30 a,b,c,d) Size, 15 to 30$\mu$ by 6 to 8$\mu$. Cells pyriform to obliquely ellipsoid. Anterior rounded. Posterior narrowed. Pharynx almost reaching the middle, but not beyond, with a crooked opening. Chromatophores, one or two, brown or green to blue-green or reddish. In cool, standing, clear water, more reddish; in fertilized waters, more blue-green. Metabolic product starch. In optical cross section only slightly higher than broad. In standing water, planktonic.

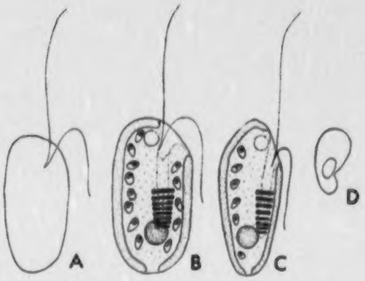


Fig. 31 *Cryptomonas ovata*
a. Outline
b. Detail of a small organism from dorsal side
c. Detail of a small organism from lateral side
d. Cross section of a small organism through gullet area

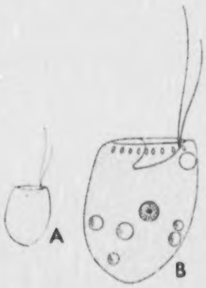


Fig. 32 *Cyathomonas truncata*
a. Outline
b. Detail

> *C. ovata* Ehrenberg: (Fig. 31 a,b,c,d) Size, 20 to 80$\mu$ by 5 to 18$\mu$ by 6 to 20$\mu$ thick. Ovate, often weakly bent, with a distinct anterior upper corner. One or two chromatophores. Pharynx reaching beyond the mid point, has a crooked opening.

*Genus Cyathomonas*: Colorless cells distinctly flattened giving the appearance from the small side of being long elliptic. Posterior round or bluntly pointed, anterior broad and obliquely truncated. The broad lateral sides (in contrast to *Chilomonas*) more twisted and equally arched. The furrow is almost wholly sunken into the pharynx. The opening of the pharynx more toward the ventral surface and extending a short distance ventralward, but its posterior portion turns suddenly toward the dorsal side where the major portion of the pharynx can be found as a twisted sacklike end. The opening of the pharynx is surrounded by an apparently closed ring of light refractive bodies (pharyngeal ring). This ring is set off from the pharynx. Pellicular layer smooth, dense, not metabolic but possesses longitudinal fibrillae. Contractile vacuole, one, in the anterior dorsal corner. Nucleus large, distinct, dorsal, somewhat posterior of the middle. Flagella body length and almost equal. Nutrition reported by some authorities (13) to be holozoic but considered by others as saprozoic.

*C. truncata* Ehrenberg: (Fig. 32, a,b) Size, 15 to 30$\mu$, sometimes smaller. Only one species which has characteristics of genus. Pharynx is difficult to distinguish on this usually active organism but the shape, antero-lateral position of flagella and especially the jewel-like band of pharyngeal ring, make identification relatively simple. They are colorless.

**Order Phytomonadida:** Sometimes also known as Volvocales. These are grass green or colorless flagellates, many relatively small. The individuals are usually rounded, but spindle-shaped, flattened, spirally twisted and other forms exist. Contains both single living and colonial forms. Except in one family (Polyblepharidae), the body is enclosed by a distinct membrane composed at least partly of cellulose. One family (Phacotidae) has a rigid membrane which may be impregnated with iron. The common number of flagella is two; they are equal. With certain species four flagella are the usual number and organisms with one to eight flagella have been assigned to this order. A single cup-shaped chromatophore is typical for the group but two or more are also reported for various species. A single pyrenoid is most common, usually posterior. Starch stored around pyrenoid and in cytoplasm. Starch storage also occurs in colorless forms. A stigma, variably placed but usually anterior, is present in a number of both the colorless and green types. Oil as a stored metabolic product occurs frequently but usually not in appreciable quantities. A red pigment occurs in some species. These organisms are most frequently found in clean streams although some have been known to occur in grossly polluted streams and in Imhoff tanks and open digesters. These green forms require light, and if this can be obtained, gross pollution has no adverse effect on many of them.

**FAMILY POLYBLEPHARIDAE:** Free living solitary types with flexible bodies (no apparent membrane). Green or colorless. The one colonial genus, *Raciborskiella,* has not been reported from sewage. Two or four flagella common, but one to eight have been reported. Kudo, following Dangeard's description, applied this family name to a group having six or more flagella. This is not in agreement with Pascher or with Hall's more recent arrangement which disagrees slightly with Pascher's selection of genera belonging to this family.

*Genus Polytomella*: These are colorless with ellipsoid or inverted pyriform cells. While a distinct cell membrane is not present, a periplast exists which may be separated from the protoplasm. The anterior bears a small papilla. When viewed from the anterior end, the four equal flagella appear rather regularly arranged in the form of a cross and they approach body length. No chromatophores. The stigma is anterior. Starch is stored in the posterior ⅗ of the peripheral portion of the body as small discs. The two contractile vacuoles are situated near the anterior end. Nucleus slightly anterior of the middle.

*P. citri* Kater: (Fig. 33 a,b,c,d) Size, 10 to 14$\mu$ (aver. 12$\mu$) by 7 to 10$\mu$ (aver. 8$\mu$). Inverted pyriform, oval or elliptical with the posterior end bluntly pointed. A papillary ridge arises from the anterior face. The thin cell membrane cannot be distinguished and the cell is very variable in shape and flexible. The optical cross section is circular. The flagella arise two on each side of the papillary ridge, each pair from a single basal granule (blepharoplast). Flagella equal and slightly shorter than body. Two anterior contractile vacuoles discharge alternately. Nucleus 3$\mu$ in diameter, with a distinct karyosome, located between the ¼ and ¾ distance, along the longitudinal axis of the cell. The karyosome is attached to the nuclear membrane by achromatic strands up to 6 in number. Approximately 18 longitudinal striations may be seen on living specimens. Stored food, starch, located peripherally, usually more plentiful in one end than in the other but may be absent. The starch granules frequently obscure the internal structure of the cell. Sexual reproduction not known. Encystment occurs. Palmelloid forms not reported. Eyespot apparently absent.

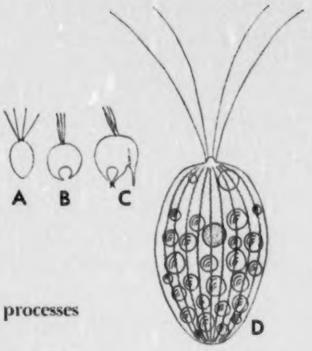


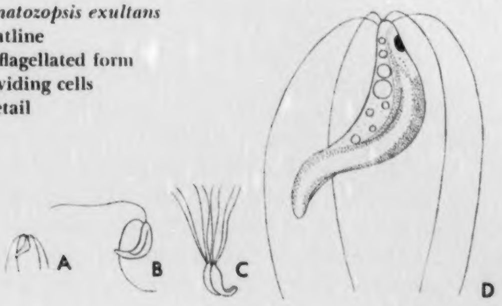
Fig. 33 *Polytomella citri*
a. Outline
b,c. Outlines of forms with posterior processes
d. Detail

*Genus Spermatozopsis*: Flagella two or four. The cells are sickle- or weakly screw-shaped but even the sickle form does not lie flat in a single plane. Anterior rounded, posterior blunt or often becoming smaller and with the end pointed. Flagella curve backward when at rest and are 1½ times the cell length. Chromatophore lies along the convex side of the cell, bowl-shaped, distinctly wider anteriorly, often twisting over toward the concave side in the posterior portion. Concave side otherwise hyaline. Two anterior contractile vacuoles. The chromatophore frequently possesses a line of small sparkling granules along its border. There are also other granules.

Cells normally fairly constant in shape but, under unfavorable environmental conditions, they may become very changeable.

*S. exultans* Korschikoff: (Fig. 34 a,b,c,d) Size, 7 to 9$\mu$. As found in nature, forms with two flagella apparently more common than those with four. Usually associated with sapropelic organisms.

Fig. 34 *Spermatozopsis exultans*
a. Outline
b. Biflagellated form
c. Dividing cells
d. Detail



**FAMILY CHLAMYDOMONADIDAE:** Organisms having a cell membrane which makes them somewhat rigid. Two, or four flagella. Most genera of the family are green but several are colorless. The cell membrane may stop short of the anterior end, may cover it except for a flagellar opening, or may entirely cover the anterior end and the flagella each emerge through pores. The family is otherwise very similar to the Polyblepharidae. Members of this family will be discussed under three groups: 1) Green forms with two flagella; 2) Colorless forms with two flagella; and 3) Green forms with four flagella.

1. Green forms with two flagella.

*Genus Chlamydomonas*: Cells spherical, pyriform ellipsoidal, or cylindrical in shape. A cell membrane of cellulose is typical in this genus and a few species have a gelatinous sheath external to the membrane. Apical flagellar insertion into a papilla is common but not universal in this genus. The chromatophore commonly cup-shaped, but two cups base to base, and more rarely other forms of chromatophores, may occur. The "cup wall" of the chromatophore may be thin or thick. In some species the chromatophore may occupy most of the protoplast. One pyrenoid is most common but two to several or none are also known. The usual number of vacuoles (2) is arranged near the flagellar origin but vacuoles vary widely in some species. Eyespot location is variable in the genus but fairly constant for a given species and for all species it is in the anterior ⅔ of the body. The eyespot is dense and circular, oval or occasionally almost linear. In the forms with cup-shaped chromatophores, the nucleus is in the cup (but surrounded by the colorless cytoplasm). In species with other than cup-shaped chromatophores, the location of the nucleus tends to be near the center of the cell, but variations from this arrangement are also known.

*Chlamydomonas* sp.: (Fig. 35 a,b) All reports of *Chlamydomonas* so listed indicate that the species was not identified. This is understandable since Pascher in 1913 listed 148 species. Many of these are differentiated on such minor variations as placement of pyrenoids. The large number of species, their rapid swimming and the slight differences between species have compounded the troubles and, as a result, most workers tend to call all small, rapidly swimming, nearly spherical, green or nearly green, organisms *Chlamy-*

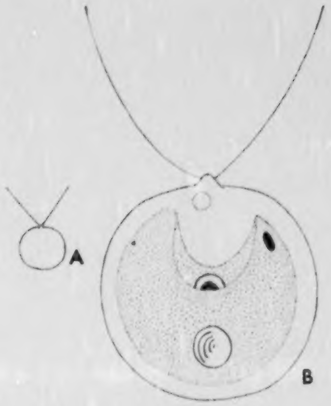


Fig. 35 *Chlamydomonas species*
a. Outline
b. Detail

*domonas.* Palmer* feels that more careful study of the chlamydomonads might yield useful information as to pollution indicators and similar data.

*Genus Chlorogonium*: Cells fusiform, spindleform or ellipsoidal. Morphologically easily confused with small euglenas. Posterior pointed, anterior often truncated or rounded. Two flagella arising on, or near, opposite borders of the anterior end. Both ends of the cells are frequently hyaline. Chromatophore varies from massive to thin, lateral, often irregular sheets. Pyrenoids, none to several. Nucleus usually central, in an indentation in the chromatophore if the chromatophore is massive. Flagella about ½ body length. Eyespot in the anterior half of the cell almost ribbonlike. Upon division, four cells are produced from mother protoplast. Transverse or oblique division may occur. The cells are retained in the mother cell membrane until fully formed. Zygotes known for some species.

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*Verbal communication.

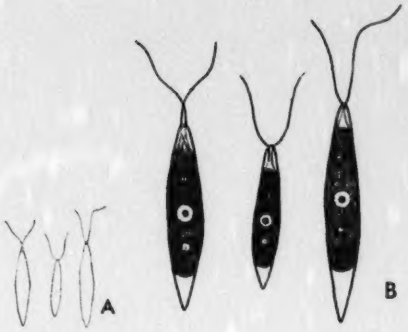


Fig. 36 *Chlorogonium elongatum*
a. Outline
b. Detail

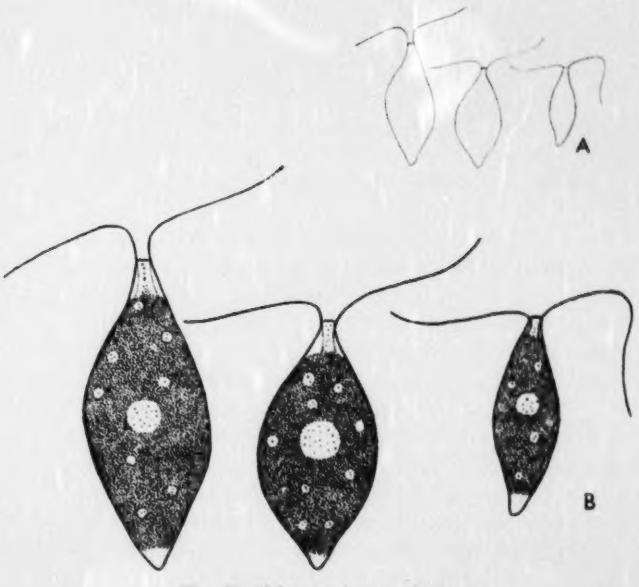


Fig. 37 *Chlorogonium euchlorum*
a. Outline
b. Detail

*Chl. elongatum* Dangeard: (Fig. 36 a,b) Size, 20 to 45$\mu$ by 4 to 6$\mu$. Cells elongated spindleform, very variable, 9 to 15 times as long as broad. Posterior end drawn out, hyaline. Anterior end elongated, bill-like, truncated, hyaline. Two equal flagella about ⅙ the length of the body are inserted on the anterior end. The chromatophore does not reach either end of the cell. In the center the chromatophore is hollowed out where the nucleus lies. A pyrenoid lies in each end about in line with the long axis. Zygotes and cysts known, palmelloid forms rare. Slightly saprobic. Pascher (14) feels that this is a poorly defined species because of the variation in morphology and the variation in the form of chromatophore. When cultured, the organism frequently becomes more like *Chl. euchlorum* in morphology, in appearance of chromatophores and number and arrangement of pyrenoids. Cell division may be transverse.

*Chl. euchlorum* Ehrenberg: (Fig. 37 a,b) Size, 25 to 70$\mu$ by 4 to 15$\mu$. More thick and plump than *elongatum*. Posterior end usually not drawn out; hence, the cell is more elongated pyriform to spindleform, than fusiform. The anterior is often very long and reduced in size forming an acute hyaline point. Chromatophore very diffuse and its morphology difficult to discern. It appears as a thick elongated mass, reduced on both ends and in which the nucleus lies in a lateral equatorial hollow. Actually, it is a very strong, thickened, flat structure rolled into an almost cylindrical shape. A large stigma is usually present. Numerous contractile vacuoles scattered throughout the protoplast and numerous pyrenoids in the chromatophore. Typically produces four daughter cells upon division. Gametes and aplanospores are formed. Palmelloid state known. Due to variations in morphology and other characteristics, this is not a well circumscribed species. A variety, *minuta,* which is under 30$\mu$ in length has been described.

*Genus Sphaerellopsis*: The broadly pyriform cell is surrounded by a wide elliptical gelatinous sheath. Sheath and cell are not the same shape and the genus differs in this regard from the envelope bearing *Chlamydomonas*. Anteriorly the cell tends to be drawn out and slightly rostrate, with a very broadly pointed posterior. The two ends are usually almost free of the envelope. Chromatophore cup-shaped, the anterior end of

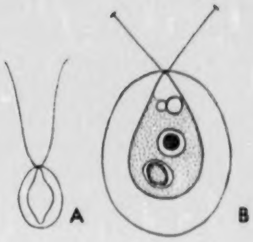


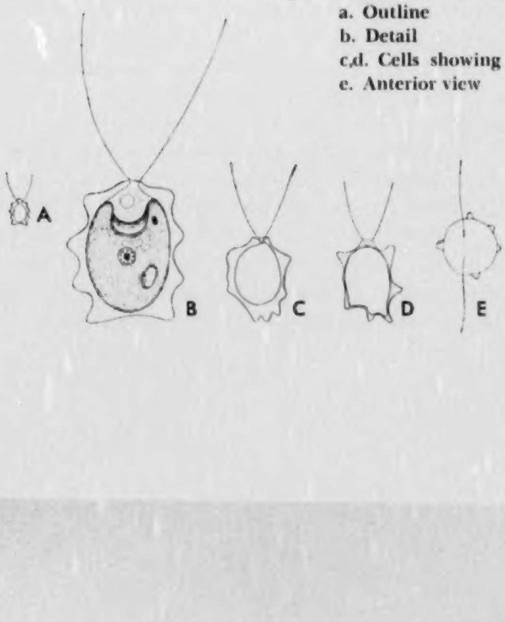
**Fig. 38** ***Sphaerellopsis fluviatilis***
**a. Outline**
**b. Detail**

the protoplast hyaline. A single pyrenoid is deeply embedded in the posterior portion of the chromatophore. An eyespot lies toward the anterior of the cell and two contractile vacuoles are just under the flagellar origin. Four to eight daughter cells are formed and develop envelopes before leaving the mother cell (differs from *Chlamydomonas* with gelatinous envelopes in this regard).

*Sph. fluviatilis* (Stein) Pascher: (Fig. 38 a,b) Size, 14 to 30$\mu$ by 10 to 20$\mu$. Anterior protoplast end reaches to the border of the envelope. Flagella do not emerge through pipelike openings. Stigma anterior. Posterior sometimes drawn out as a slender process which may be bent. Flagella, two; ½ to equal body length.

*Genus Lobomonas*: Similar to *Chlamydomonas* but having blunt irregularly arranged processes on the cell wall. Chloroplast cup-shaped with an eccentric posterior pyrenoid. The

**Fig. 39** ***Lobomonas rostrata***
**a. Outline**
**b. Detail**
**c,d. Cells showing variations in morphology**
**e. Anterior view**



eyespot a thin ribbon in the anterior half of the cell. Flagella two, about body length. Cell in optical cross section never distinctly round but often weakly blunt cornered. Protoplast with the typical construction of the chlamydomonads, also typical chromatophore, pyrenoid and stigma. Two or more contractile vacuoles; when two only, located at base of flagella; if more, then scattered.

*L. rostrata* Hazen: (Fig. 39 a,b,c,d,e) Size, 5 to 12$\mu$ by 4 to 8$\mu$. Cells inverted pyriform to pear-shaped, less frequently elliptic; membrane of actively growing cell with thick, irregularly arranged, broad cone-shaped and blunt, wartlike protuberances. Protoplast usually almost ellipsoid. Young cells with delicate membranes. Protoplast then with a more or less wavy contour from which the thicker membrane differentiates. Anterior has a sharply offset, perpendicularly truncated, wartlike papilla. Flagella somewhat over body length. Chromatophore is bowl-shaped and reaches to the base of the anterior papilla. Pyrenoid usually somewhat lateral and in the posterior half of the cell. Nucleus almost central or somewhat lateral and more posterior. Stigma a short rod, in the anterior third. Two contractile vacuoles, anterior. Daughter cells, 2-4-8, formed by transverse division. Spores or zygotes unknown.

2. Colorless forms with two flagella.

*Genus Polytoma*: Members of this free swimming, solitary, biflagellated genus are in many cases identical to some of the *Chlamydomonas* except that chromatophores are lacking. The usually dense membrane may be smooth or longitudinally striated and may sometimes be overlaid by a gelatinous covering. The membrane may have a yellow or light brown color. Contractile vacuoles, usually two in number, lie at base of the equal, approximately body length, flagella. The flagella usually arise from an anterior papilla but this is not always present. A very noticeable feature of members of this genus is the presence of starch granules which may pack the cell but are often more concentrated in one end—usually the posterior. Droplets of yellow-brown to reddish oil may also be present. Since the organisms lack chromatophores, pyrenoids are also absent. The nucleus lies in the body axis and usually in the anterior half. Sexual reproduction with zygote formation known.

*P. uvella* Ehrenberg: (Fig. 40 a,b,c) Size, 15 to 30μ by 9 to 20μ. Shape very variable but remaining constant in a given strain. Pyriform, ellipsoid pyriform to almost elliptical, but sometimes almost pyriform-spherical. Never spherical or elongated-cylindric; however, sometimes very elongated pyriform. Posterior rounded, occasionally reduced and almost pointed, or, very rarely, drawn out into an elongated tail. Membrane usually delicate, sometimes thickened posteriorly (or possibly the cytoplasm pulls away from the membrane in the posterior area) and distinguishable only on sides near the anterior. It is ordinarily smooth but may occasionally show indistinct longitudinal striations. The organism may or may not have an anterior papilla. In its natural environment it commonly possesses a gelatinous outer covering. Flagella 1 to 1½ times body length. Elliptical, somewhat flattened, starch granules frequently lying near the periphery and often localized in one end—most usually the posterior. Nucleus ordinarily anterior of the middle. Stigma usually present but may be absent. If present, located in the anterior third and lateral in position. Very variable in shape and color. Two anterior contractile vacuoles. Division into four daughter cells may be transverse. A very cosmopolitan species often found in waters where decomposition is active.

3. Green forms with four flagella.

*Genus Carteria*: Cells solitary. Distinguished from the otherwise completely similar *Chlamydomonas* by the possession of two pairs of flagella that diverge from each other at an angle of 90 degrees. Cell form; round, pyriform, ellipsoid, to cylindrical and all variations between. In optical cross section most usually round, never distinctly flattened, though one species is somewhat compressed while others possess a blunt four-cornered anterior end. Membrane delicate to dense, never shell-like. With or without papillae. Chromatophore essentially cup-shaped but also, in many, diverging into modified forms. This very seldom occurs in the better known fresh water species and only in one is the chromatophore divided into discs. Pyrenoids none, one or two. With, or rarely without, stigma. Two anterior contractile vacuoles. Reproduction by longitudinal division or sometimes by apparently transverse division. Usually four daughter cells produced, which often are somewhat varied

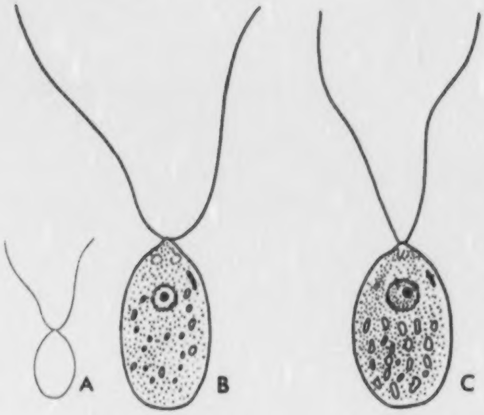


**Fig. 40** ***Polytoma uvella***
**a. Outline**
**b. Detail**
**c. Detail of organism with a large amount of stored starch**

in form as compared with the adult cell. Extreme heterogamy not known as in *Chlamydomonas*. Palmella state and non-zygote cysts very probably exist, but have been observed in a few species only. Pascher lists 33 species and considers this genus to be too inclusive. Several other genera contain four-flagellated organisms but do not show the characteristic morphology of this genus.

*C. globosa* Korschikoff: (Fig. 41 a,b) Size, 18 to 28$\mu$. Cells exactly spherical. Membrane delicate and without anterior papilla. Flagella approximately body length or somewhat longer. Chromatophore smooth with basal thickening. The posterior portion of the chromatophore extends to the middle or beyond and provides a flat bottom to the lumen. Uniform thickenings reach upward along the cell wall to base of the flagella. A single pyrenoid lies axially embedded in the basal portion of the chromatophore. Stigma close to the middle, or somewhat higher; granular, irregularly shaped, becoming large. Nucleus in anterior half of the cell. Contractile vacuoles two, located anteriorly. True spores not reported. Zygotes and palmella forms known.

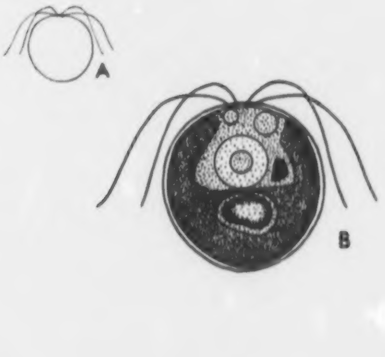


**Fig. 41** ***Carteria globosa***
**a. Outline**
**b. Detail**

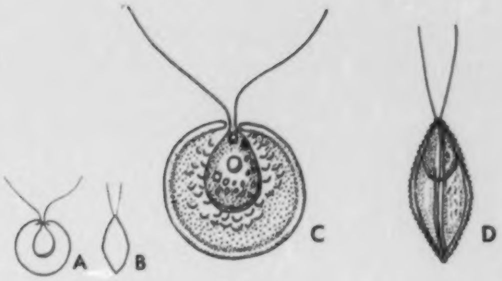


Fig. 42 *Phacotus lenticularis*
a. Outline, dorsal view
b. Outline, lateral view
c. Detail, dorsal view
d. Detail, lateral view

FAMILY PHACOTIDAE: Having a covering composed of two watchglass-like halves, reminding one of a shell of a clam. This shell-like covering frequently contains large quantities of calcium and may be brown due to impregnation with iron. The protoplast is usually compressed and in some cases is in contact with the central portion of the shell, or the protoplast may even fail to approach filling the central portion of the shell and be surrounded with a gelatinous material. Such gelatinous material always occupies the border area of the shell. Under certain conditions, and especially when the protoplast has divided, there may be a considerable increase in the volume of the gelatinous matter and the two halves become widely separated. The shells are round, round oval or elliptic. Two apical flagella. The chromatophore has the typical cup-shape common to the chlamydomonads. Palmella forms known in some species. Reproduction following fusion of gametes apparently rare. Simple division into four to eight cells followed by release from gelatinous matrix of mother cell is the usual manner of multiplication.

*Genus Phacotus*: Free swimming cells not filling the shell but which touch it only at the anterior end. Space between shell and protoplast and the margins of the shell filled with a thin gelatin-like substance. The shells are circular, and the shelled organism appears elliptical when viewed from above. The protoplast is either ovoid or compressed ovoid. Usually two contractile vacuoles near the origin of the two flagella. Chromatophore large, thick and cup-shaped with one or more pyrenoids.

Four daughter cells are usually formed by simple division. The daughter cells may be retained within the mother cell gelatin and again divide leading to the formation of a palmella stage.

*P. lenticularis* Ehrenberg: (Fig. 42 a,b,c,d) Size, 13 to 20μ. The cell with its shell halves has a distinctly lens-shaped appearance. There is a slight separation of the two halves, or more rarely the edges of the two halves are in contact. Shell sculptured by hexagonal pits (sometimes with small punctae, sometimes entirely smooth) that give shell the appearance of having scales sometimes more striped. This pitting is distinctly noticeable at the edges of the shell. There are variations that should perhaps be classified as separate species. Shell often brown colored. Protoplast distinctly smaller than the shell, often with a bill-like attenuation of the anterior end. Chromatophore cup-shaped and with the pyrenoid embedded in the thickened posterior portion. Stigma in the posterior half. Nucleus anterior of middle. Contractile vacuoles in the hyaline zone, which lies under the anterior papillae from which the two body length flagella spring. Division typical of the family. Shells often dark brown and almost opaque, making the organism an easily overlooked species. (A recent article (a) assigned *Phacotus* to the group of "clean water algae." The inclusion of this organism in the present writing as a polluted stream form is based on its being "most abundant in polluted zones" of the Scioto River (b).)

FAMILY SPONDYLOMORIDAE: Colonial form usually with 16 cells but 2, 4, and 8 cell colonies known. All cells in colonies with orientation in the same general direction. Not bound together by a gelatinous covering and individual cell without gelatinous envelope. Circlets of 4 cells (or 2 cells); arranged with the cells of one group alternating with the cells of the adjacent group to yield close packing. Cells with 2 or 4 flagella. Chromatophores usually typically chlamydomonad-like.

*Genus Spondylomorum*: Colonies usually with 16 loosely united cells when fully developed. Cells may be irregular in shape. Normally four overlapping circlets of 4 cells each arranged so

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(a) Palmer, C. M., Tarzwell, C. M. and Walter, H. J. "Algae of Importance in Water Supplies." *Pub. Wks. 86* (6) 107, (1955).

(b) Lackey, James B. "Protozoan Plankton as Indicators of Pollution in Flowing Streams." *Pub. Health Repts. 53*, 2037-2058, (1938).

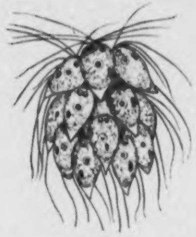


Fig. 43 *Spondylomorum quarternarium*

that the cells of one circlet are set in the grooves of its adjacent circlet. Anterior ends of cells somewhat converging. Posterior ends diverging from the axis of the colony but posterior circlet shows little divergence. Colonies frequently irregular such as being made up of only two circlets or circlets containing only two cells. Cells barely touching and joined at point of contact but lacking gelatinous covering on either cells or colony. Cells inverted pyriform to ellipsoid and somewhat bellied outward on the inner side of colony. Posterior drawn out and with protoplast somewhat pulled away from the cell wall of this region. Chromatophore cup-shaped and does not possess discernable pyrenoids. Nucleus central. Stigma short to long, line-shaped; in the middle or posterior half of cell. Two anterior contractile vacuoles. Flagella, 4 per cell; they lie toward the outer side of the colony (not apical) and reach laterally; and are 1½ times

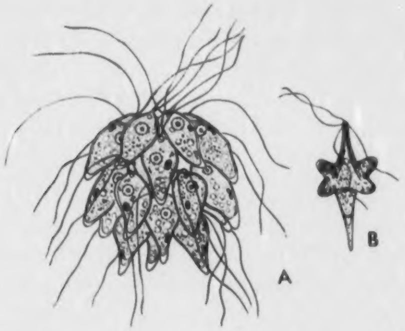


Fig. 44 *Pyrobotris gracilis*
a. Adult colony
b. Embryonic single cell form (*Chlorobrachis gracillima* state)

the cell length. Colonies containing daughter cells within mother cell may sometimes be seen.

*S. quarternarium* Ehrenberg: (Fig. 43) Cells 10 to 20μ (also 26) long, 8 to 15μ broad. Colonies up to 50μ long. Similar in all respects to the genus description.

*Genus Pyrobotrys* (Chlamydobotrys—Pascher): Silva and Papenfuss (17) have recently reviewed this genus and concluded that *Pyrobotrys* is the more valid generic name and that *Chlorobrachis gracillima* is the zygote stage of one of the species of this genus. Similar to *Spondylomorum* but cells with 2 flagella. Colonies of 8 or 16 cells. Irregular colonies frequent. Cells pyriform, inverted pyriform or thick spindle-shaped. Anterior often reduced and bent. Protoplast adheres closely to cell membrane except in the somewhat drawn-out distal portion where it may be loosened. Chromatophore cup-shaped, sometimes lightly longitudinally striated. Pyrenoid lacking. Possessing a large stigma. Nucleus in the anterior half of the cells. Daughter colonies form asexually within the cells of the mother colony. Sexual reproduction also known.

*P. gracilis* Korschikoff: (Fig. 44 a,b) Cells up to 20 or 25μ long. Colonies up to 44μ long. Cells inverted pyriform or thick spindle-shaped. Colonies usually 16 cells and generally composed of circlets of 4 cells each. Posterior of cells not distinctly pointed or papillalike. Protoplast may be distinctly separated from the membrane in this region. Flagella 1½ to 2 times cell length and may arise from anterior papilla. Chromatophore not thickened in the basal portion and lacking pyrenoid. Nucleus in anterior half. Stigma in in the middle or posterior half. *Chlorobrachis gracillima* is the zygote stage of this organism according to Strehlow (19) and to Behlau (1).

FAMILY VOLVOCIDAE: Chlamydomonad-like cells united into platelike or approximately spherical colonies. Cells with gelatinous envelope, or colonies with gelatinous matrix, covering, and often extending well beyond, cells. Cells with two flagella. Cells united along flattened sides or with cell processes extending between cells. Both sexual and asexual reproduction exist in most genera. The stages in the development of the colonies are discussed by Hall (4), and it is recommended that the interested reader consult that writer.

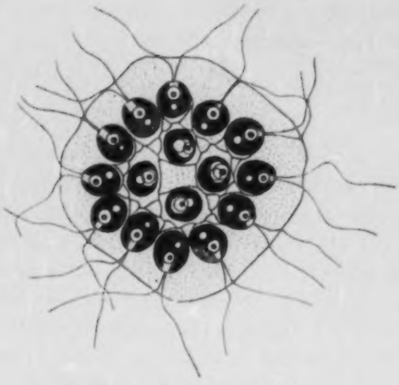


Fig. 45 *Gonium pectorale*

*Genus Gonium*: Colonies of 4 to 16 cells in flat plates one cell in thickness. Individual cells frequently connected by processes formed by the pushing out of the cell walls which flatten at the point of contact. The whole colony is enclosed by a square, thickened, platelike, gelatinous matrix. Colony plates are actually slightly curved. Chlamydomonad-like cells with two flagella which all reach in the same general direction but the peripheral cells are tipped outward and the flagella of these cells appear to reach almost directly outward from the margins of the plate. Actually these flagella are directed slightly toward the sides of the plate from which the flagella of the central cells emerge; i.e., the convex side. The protoplast is usually pulled away from the cell membrane except at the anterior end. Some species have a papilla from which the flagella emerge. The flagella each pass through a tubelike opening in

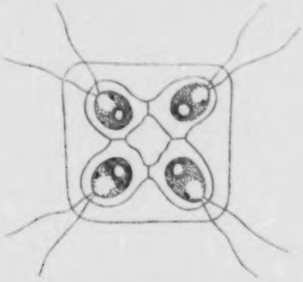


Fig. 46 *Gonium sociale*

the gelatinous coating, to reach the exterior. The flagella are approximately twice the cell length. The chromatophore is cup-shaped and the basal thickening reaches to the approximate midpoint. Stigma is near the anterior border of the chromatophore. Two contractile vacuoles adjacent to bases of flagella and having definite orientation to the flagella. The nucleus is within the lumen of the chromatophore and thus in the anterior half of the cell. A very large pyrenoid occupies the basal portion of the chromatophore and causes an upward bulging of the bottom of the lumen. Sexual and asexual reproduction occur. Sexual reproduction by fusion of similar or dissimilar gametes. A thick walled zygote is produced which, on germination, releases four cells. Each cell produces a colony in the same manner as colonies are produced asexually in the cells of the colonies.

*G. pectorale* Müller: (Fig. 45) Cells 5 to 14$\mu$ long. Colonies up to 70$\mu$ (also 90) in diameter, most usually 16 cells, seldom 8 or 4 cells. Cells ellipsoid to weakly pyriform with posterior rounded and anterior only slightly reduced in size or often practically spherical. Cells lie closely adjacent to one another in the colony. Chromatophore with a thick, often upwardly convex, posterior portion in which is embedded the pyrenoid. Nucleus is in anterior half. Lumen of the chromatophore usually somewhat flask-shaped. Stigma large, located anteriorly.

*G. sociale* (Dujardin) Warming: (Fig. 46) Cells 10 to 22$\mu$ by 6 to 16$\mu$. Colonies 4 celled, never 16 celled. Cells distinctly pyriform, 1½ to 2 times as long as wide, and with protoplast pulled away from cell membrane over the entire surface. Gelatinous envelope of individual cell often distinct, common gelatinous covering frequently difficult to discern. Flagella, two, usually 1½ times cell length often arising from a papilla. A widely distributed species but usually not abundant. It is easily confused with four-celled colonies of *G. pectorale*. Pascher believes it to be somewhat more catarobic than *G. pectorale*.

*Genus Pandorina:* A colonial form usually containing 16 cells which are arranged as a short ellipsoid with rounded ends, or often almost spherical. The cells are radially arranged and generally close packed, being flattened along the line of contact so that the posterior portion of the cell is pyramidal. The

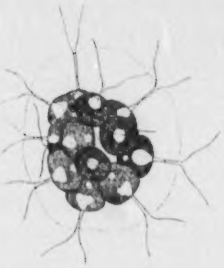


Fig. 47 *Pandorina morum*

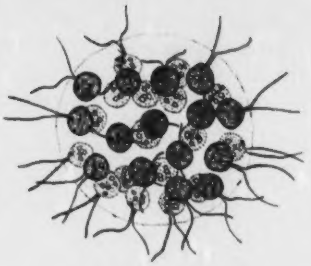


Fig. 48 *Eudorina elegans*

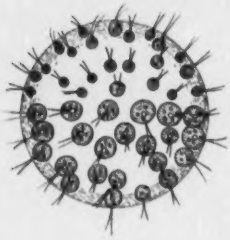


Fig. 49 *Pleodorina californica*

cells may be somewhat separated and rounded up but usually, when in this condition, show daughter colonies within the cells of the mother colony. The colony is covered by a gelatinous envelope which may be thin but is often 2 to 3 times the diameter of the ball of cells. The posterior end of the cell does not come to a point but is rounded off and the chromatophore occupies most of it. The anterior face is flatly rounded and the chromatophore underlies this face as a thin extension, almost converging at the bases of the flagella. A small, practically spherical, lumen remains which is usually wider than deep. The stigma is on the anterior face. Several pyrenoids or a single large pyrenoid may be present in the thick basal portion of the chromatophore. Sexual reproduction is known, but asexual reproduction is encountered almost exclusively.

*P. morum* (Müller) Bory: (Fig. 47) Cells 9 to 16μ long. With the characteristics of the genus.

*Genus Eudorina*: An ellipsoid to almost spherical colony of 32, or occasionally 16, cells. Cells widely spaced and sometimes in plates or circlets. The anterior and posterior circlets usually contain 4 cells each and the 3 median circlets 8 cells each. It is sometimes difficult to find a colony oriented properly to make the plates readily visible. Cells are usually near the periphery of the gelatinous envelope of the colony and the two flagella reach outward in a radial fashion. The two flagella pass through the gelatinous envelope in a single tubelike structure. Chromatophores chlamydomonad-like. The posterior portion of the colony envelope is frequently truncated and possesses liplike or low, lobelike projections. The stigmas of the cells gradate from large in the anterior circlet to small in the posterior circlet. Sexual and asexual reproduction.

*E. elegans* Ehrenberg: (Fig. 48) Cells 16 to 24μ long. Colonies 60 to 200μ long. Cells spherical (not broader than long) and chromatophores without distinct longitudinal striae. With one or several pyrenoids. Lumen of chromatophore sack-shaped. Basal portion of chromatophore only slightly heavier than lateral portion. Stigma near base of flagella. Smith (18) states that the envelope of the colony of this species is round and without a posterior protuberance.

*Genus Pleodorina:* Colonies with 32, 64 or, most usually, 128 cells. The cells of the anterior half are noticeably smaller than those of the posterior half. All cells are practically spherical and are located very close to the periphery of the colony. The smaller ones in the anterior half have a prominent stigma and one pyrenoid, while those in the posterior half may lose their stigma, and usually possess several pyrenoids. The flagella are 1½ to 2 times as long as the cell diameter and emerge to the exterior through a tubelike passage in the gelatinous envelope of the colony. Cells of the posterior region are reproductive cells for both sexual and asexual reproduction while anterior cells are purely negative in function.

*P. californica* Shaw: (Fig. 49) Cells, anterior 13 to 15μ with smallest cells nearest anterior pole, posterior 27μ approximately. Colony 250μ. Vegetative cells extend practically to colony equator.

**Order Euglenida:** There is perhaps no other group of protozoa which shows such extremes as this group. This order contains all of the very large flagellates encountered in sewage treatment and polluted streams, but also included are some very small forms (example, *Scytomonas*). It has many genera which are green and are in this sense strictly plants, but there are also a great many colorless forms that are animal-like. Many of the green forms appear to thrive in waters of high organic content, and it has been demonstrated that nitrogen in the form of amino acids is used by some. Saprophytic nutrition is, therefore, known both in some green and some colorless forms. Strict holozoic nutrition is also thought to exist, and even in predominantly holozoic forms, paramylum (a starchlike storage product) is produced and stored as it is in the green forms. Some species are able to photosynthesize their food in light, or to lead a saprophytic colorless existence in dark. Many species are spindle-shaped or near spindle-shaped, others flattened, while very diverse types of morphology are also present. Characteristic of many members of the group is a contracting, expanding, twisting, turning, creeping motion that is often referred to as metaboly and, since it is characteristic of many members of this group, it is also known as euglenoid movement. Again, in contrast, many swim freely and have a nearly constant shape but a flexible body. Then, too, many are quite rigid and sometimes creeping movement may be due entirely to flagellar action. The typical and probably universal structure in the order is a gullet reservoir or flasklike invagination of the anterior end. In some members of the order, this gullet-reservoir, also known as the cytopharynx, is difficult to see; especially when stored food or chromatophores obscure the structure. In the holozoic forms this structure is reported to act as an organelle of food intake; however, this is a matter of considerable controversy. There is no indication that nutrients enter the cell specifically through the cytopharnyx in the holophytic forms. The contractile vacuoles are in the protoplasm near the bottom of the cytopharynx; one or two is the usual number. These discharge into the swollen or reservoir portion of the gullet-reservoir, and it is common for the primary contractile vacuole to form from the fusion of smaller secondary vacuoles. Organisms with one flagellum are most common but many with two flagella occur, while a few species have no visible flagella. The flagellum is inserted near the bottom of the reservoir and emerges through the gullet. Helical striation of the pellicle layer is common. Sometimes other markings replace the striations but the helical arrangement usually remains. Paramylum and oil are the stored food reserves. Paramylum, although starchlike, does not yield the blue color with iodine. As the readily apparent stored food, it takes on distinct shapes such as rings, links, discs, rods, or bricks. The form of the paramylum bodies is sometimes characteristic even of species and, therefore, useful in differentiating groups. Some of the paramylum is apparently formed at the pyrenoids. Chromatophores, if present, have usually one pyrenoid each. Within the genus *Euglena* pyrenoids may be used as a basis for subdivision. If a single chromatophore is present, it may contain several pyrenoids.

Recent authors do not agree concerning the subdividing of this order, but it is apparent that some older systems were rather artificial and based on differences that are now known not to exist. Hall (4) has been followed in most of this discussion and he divides this group only to suborders. Familial divisions are difficult to make and even more difficult to delimit, so the wisdom of Hall's handling must be acknowledged and the genera listed only under the suborder. One must hasten to point out, however, that research may, in the near future, shift some genera to suborders other than those in which they are listed.

**Suborder Euglenoidina:** This suborder contains all of the green euglenoids. In addition to the green forms, closely related colorless forms are included. This suborder has only swimming flagella (i.e., no member has trailing flagella) and in all except two families only one flagellum is present. The sheath of the flagellum is not thickened near the body. Some species are rigid and some metabolic.

*Genus Euglena*: Cells, long cylindrical, spindle-shaped, needlelike or pyriform. Many are very metabolic and usually creep on the substrate, others may be more rigid. Many swim freely. The genus contains green forms, but the color may occasionally be lost. The anterior end is usually obliquely truncate with a small central or slightly lateral depression where the pellicle invaginates into the gullet-reservoir and from which the single flagellum emerges. Most show striated markings. They have chromatophores and a stigma or eyespot. The stigma is composed of numerous red granules and is located at the anterior of the body. The flagellum is single but bifurcates; the two roots are demonstrable only in stained specimens. A few species have no visible flagellum. The pellicle is striated in most of the species, and subpellicular bodies which stain with neutral red may be demonstrated in many species. Both structures usually show helical arrangement. Radial symmetry is almost universal.

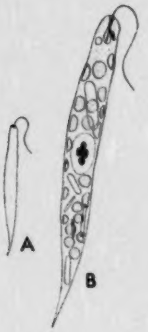
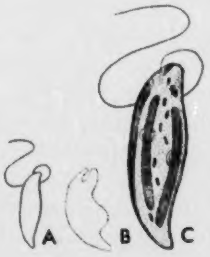


Fig. 50 *Euglena acus*
a. Outline
b. Detail

Certain species may be distinctly flattened. Pyrenoids distinguishable in some species, but cannot be found in others. The pyrenoid may be sheathed with paramylum in some species, but lack a sheath in others. Metaboly is common, but some forms remain quite constant in shape. Both creeping and swimming motions are found in the group. Paramylum and, to a lesser extent, oil are the stored food materials. For a more detailed description of the genus, the reader is referred to *Genus Euglena* by Gojdics (3), from which the following species descriptions have been freely abstracted. While this was being written, a copy was received from Dr. E. G. Pringsheim of his "Contributions Toward A Monograph of the Genus Euglena" (15). His approach appears more logical than that of Gojdics but, since his works will not be as readily available to most American workers as will the works of Gojdics and because Gojdics also gives a key for classifying Euglenas, her classification is fol-

Fig. 51 *Euglena agilis*
a. Outline
b. Outline of an organism in active metaboly
c. Detail of *E. agilis*.



lowed here. No serious student of this group should overlook Dr. Pringsheim's analysis of the Euglenas, however.

*E. acus* Ehrenberg: (Fig. 50 a,b) Size, 60 to 310$\mu$ by 7 to 28$\mu$. A long cylindrical form with anterior tapering slightly, then reducing rather sharply and rounded, and with the posterior taper beginning near the reservoir and ending as a long point. The species might be more aptly described as fusiform or needlelike. The striae of the pellicle are usually seen only with the oil immersion and run almost longitudinally. Many disc-form, peripherally lying chromatophores which do not possess distinguishable pyrenoids are present. Paramylum in the form of rods which are usually long and narrow (5.6 x 3.4$\mu$ to 30.6 x 5.6$\mu$) and rather few in number (up to 16). Flagellum 1/6 to 1/4 body length. Swimming motion straight forward and rotating. Appears rather stiff but can bend and occasionally form an S-shape. It may be very metabolic in water where vegetation is actively decaying. Eyespot relatively small. No cysts known. There are several varieties, one of which shows ridging. Similar colorless forms may exist in rich organic infusions.

*E. agilis* Carter: (Fig. 51 a,b,c) Size, 18 to 33$\mu$ by 6 to 12$\mu$. Synonym: *E. pisciformis* (this name is preferred by many workers). This organism is quite metabolic; perhaps its most common shape is a short cylinder, bulging in the middle, somewhat rounded at the anterior end and broadly pointed at the posterior end. There is only a slight reduction to the rounded anterior, and the posterior comes to a point. The faint helical striation is not usually visible at high-dry magnifications. The chromatophores are usually two, located on opposite sides of the cell in the form of elongated, widened plates which appear as bands in lateral view. Careful examination will reveal lobed margins on the chromatophores. Each chromatophore has a rather large pyrenoid sheathed with paramylum. Some paramylum bodies not associated with the pyrenoid are in the form of very short rods. Flagellum 1 to 2 times body length. The eyespot is faint, yellowish and relatively large. It is an active swimmer. Dr. Pringsheim* disagrees with Miss Gojdics' choice of name and feels that *pisciformis* should be retained.

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* Verbal communication.

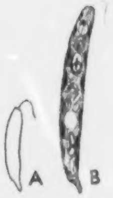

Fig. 52 *Euglena deses*
a. Outline
b. Detail

*E. deses* Ehrenberg: (Fig. 52 a,b) Size, 56 to 180μ by 7 to 22μ. Cylindrical or sometimes flattened with rounded anterior and a somewhat offset, bluntly pointed, posterior. The striation of the pellicle probably will not be seen at high-dry magnification. Subpellicular bodies which stain with neutral red underlie the pellicle and are irregularly placed. The chromatophores are lens-shaped and 26 to 34 are present. A naked pyrenoid is present in each chromatophore. The numerous paramylum bodies are brick-shaped with rounded ends and a longitudinal groove on the broad face, but unless study is careful, they may appear to be rod-shaped cylindric or oblong. The usually retracted flagellum is about 1/6 the body length. The typical nucleus is centrally located. This *Euglena* rarely swims freely but lies quiescent on the substrate or exhibits metaboly. Cysts known (cf. *E. intermedia*).

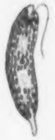

Fig. 53 *Euglena fusca*
(X 100)

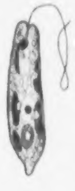

Fig. 54 *Euglena gracilis*
(X 100)

*E. fusca* (Klebs) Lemmermann: (Fig. 53) Size, 153 to 198$\mu$ by 17 to 25$\mu$. This flattened ribbonlike species has a rounded anterior and a posterior with a distinct tail piece. It is a brownish organism with oblique rows of raised wartlike projections of definite shape but this shape varies with location on the body. The extreme anterior end is free of such markings. The very numerous, small, ovoid chromatophores are very closely set but may be difficult to distinguish because of the brown color of the pellicle. Paramylum in the form of two large links, one of which is usually anteriorly and the other posteriorly located, both quite noticeable, and smaller links which vary in size may be scattered throughout the cytoplasm. The flagellum is ⅛ body length. The eyespot is dark red. Cysts have not been seen. It moves slowly, sometimes twisting, but is not extremely metabolic. It frequently exhibits a swaying motion with the tail as a pivot.

*E. gracilis* Klebs: (Fig. 54) Size, 30 to 68$\mu$ by 6 to 18$\mu$. This is probably the most commonly reported species. It is some times colorless and, if so, it may be classified as *Astasia longa* Pringsheim. There have, however, been numerous reports of the loss of chlorophyll by this species when grown in the dark in a rich medium. It is practically circular in cross section with a rounded anterior (a variety with truncated anterior has been described) and with a rounded or a short blunt and sometimes slightly set-off posterior point. There are usually 7 to 10 chromatophores but occasionally as many as 14. These may be distorted by contact with their neighbors. They are circular or ovoid, yellowish-green and peripheral. The pyrenoids are located in the center of the chromatophores and are sheathed with paramylum. Paramylum, free in the cytoplasm, is in the form of elliptical bodies which sometimes have depressed centers. These may be so numerous as to cause irregular bulging of the pellicle. Flagellum usually body length but varies from ⅛ to somewhat over body length. The cup-shaped eyespot is definitely granular and red. Distinctly metabolic but more frequently seen swimming freely with a noticeable zig-zag motion. Cysts not reported.

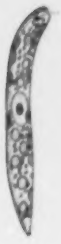

Fig. 55 *Euglena intermedia*

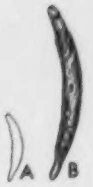

Fig. 56 *Euglena mutabilis*
a. Outline
b. Detail

Fig. 57 *Euglena oxyuris*
(X 100)

*E. intermedia* (Klebs) Schmitz: (Fig. 55) Size, 93 to 124μ by 9 to 12μ. This organism is almost constant in width over most of its length. It has a rounded anterior, a posterior 1/5 that tapers somewhat, and a cylindrical tail piece with a rounded tip. This euglena is circular in cross section. The body striae are probably more difficult to discern than in any other euglena likely to be encountered. The neutral red staining bodies are variable in number and irregularly scattered but more numerous in anterior and posterior ends. Chromatophores, about 35, 8 to 10μ in diameter, but sometimes with as many as 60 small ones. They are discoid or lens-shaped and usually lie close to the cell membrane. Paramylum, rod-shaped, and also sometimes brick-shaped. Flagellum only about 1/6 body length. Eyespot reddish-purple. Nucleus approximately central. Cysts not known. Distinctly metabolic. May attach by tail.

This species is very similar to *E. deses* in size and morphology. The most significant differences (which may all be difficult to determine on active organisms) are: the number of chromatophores, whether the chromatophores have pyrenoids and the difference in the large brick-shaped paramylum bodies which, in this species, have at most only a depressed center.

Two other varieties have been described which are differentiated chiefly by differences in paramylum bodies.

*E. mutabilis* Schmitz: (Fig. 56 a,b) Size, 80 to 125μ by 4 to 11μ. This is a long, slender, practically cylindrical euglena which tapers toward both ends. The anterior rounds off, and the posterior tapers almost to a point before rounding off. The striae of the pellicle probably will not be seen except with oil immersion and are arranged in a steep helix. The cytoplasm contains small, noticeable, randomly arranged, spherical granules. Chromatophores are usually 4 in number but may vary from 2 to 8. They are almost rectangular and are rolled into open cylinders (about 2/3 of a circle) which lie close under the pellicle. Their color is yellowish-green or green. The individual chromatophores are often difficult to discern. Each chromatophore contains one pyrenoid or very rarely two. Pyrenoids are naked. Paramylum, present as short rectangular rods, is scattered through the central cytoplasm. The flagellum is probably never observed but has been demonstrated to exist wholly within the gullet of the organism. The nucleus is central. The organism moves by a rather graceful bending and twisting and may bend itself in smooth arcs. It does not exhibit the usual expansion and contraction of the euglenoid motion. It moves by slowly lashing back and forth in a wavy manner, but in a straight line direction. It moves almost constantly. Common in iron seeps and acid water.

*E. oxyuris* Schmarda: (Fig. 57) Size, 95 to $500\mu$ by 16 to $46\mu$. This somewhat flattened euglena is twisted so that the cell has an almost cylindrical appearance. The posterior ¼ to ⅓ tapers toward a somewhat drawn-out tail piece. The anterior is rounded or has a slightly truncated appearance. The body twist is to the left and the striae, which are usually apparent, parallel the twist. May or may not possess subpellicular bodies other neutral red-staining granules. The peripheral chromatophores are oval, very numerous and with no discernible pyrenoid. One large link of paramylum in each end of the cell is typical but as many as 12 to 20 linklike bodies have also been reported. In addition to these large, conspicuous paramylum bodies, smaller links are scattered throughout the cytoplasm. The flagellum is about ⅛ the body length and is active. The loosely granular eyespot is blood red. The nucleus is centrally located. Cysts have not been reported. Movement only slightly metabolic, bending and rotating chiefly. A variety 55 to $86\mu$ long with longitudinal striations and a more abruptly tapering tail has also been reported.

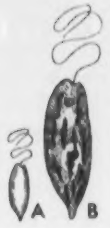

Fig. 58 *Euglena polymorpha*
a. Outline
b. Detail

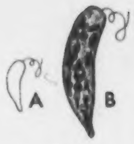

Fig. 59 *Euglena sociabilis*
a. Outline
b. Detail

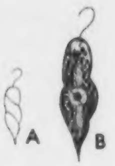

Fig. 60 *Euglena tripteris*
a. Outline
b. Detail

*E. polymorpha* Dangeard: (Fig. 58 a,b) Size, 57 to 92μ by 15 to 25μ. This is a fat, spindle-shaped organism with a rounded anterior and a posterior with a short tailpiece. The pellicle has distinct spiral striae. There are small, neutral red-staining spheres scattered at random in the cytoplasm. Chromatophores, 12 to 20 (very rarely more), are plates with irregularly lobed margins and large sheathed pyrenoids. These chromatophores are usually observed in lateral view (of the chromatophore) and have the appearance of elongated spindles with the pyrenoid bulge near the center. Paramylum, which is free in the cytoplasm, is in the form of small links. The flagellum is ½ to 2 times body length. The eyespot is coarsely granular and crimson. The nucleus is central to slightly anterior. Cysts lack a tough membrane, having a gelatinous envelope instead. Movement is typically metabolic. One of the most cosmopolitan of the euglenas. Meso-to oligosaprobic.

*E. sociabilis* Dangeard: (Fig. 59 a,b) Size, 60 to 85μ by 15 to 25μ. Nearly cylindrical to somewhat spindle-shaped. Anterior is rounded and posterior pointed without distinct tailpiece. It is distinctly striated. When the organism is stained with neutral red, small spherical granules appear randomly scattered through the cell cytoplasm, but are more numerous in the anterior portion. It contains 10 to 15 irregularly lobed, platelike chromatophores of light green color. Laterally viewed, the chromatophores have the appearance of bands. Each has a sheathed pyrenoid, centrally located. The chromatophores are closely adjacent to the cell membrane. Paramylum not associated with the pyrenoids is in the form of small rectangular links. The stored food is reported never to be abundant. Flagellum variously reported as ⅛ to ½, ⅓ to ¾, and slightly over body length. The eyespot is large, crimson and compact. The nucleus is usually located posterior to midpoint. A spherical sticky cyst is formed. The movement of the organism is typically metabolic. When at rest on the substrate, it usually takes the form of a "C" and may be attached by threads from the posterior tip.

*E. tripteris* (Dujardin) Klebs: (Fig. 60 a,b) Size, 60 to 190$\mu$ by 8 to 23$\mu$. In gross outline, this organism varies from nearly cylindrical to somewhat spindle-shaped. The anterior is round and the posterior has a drawn-out and usually pointed tailpiece. The cross section is triangular and the body is twisted to the right so that three keels ordinarily intersect a line drawn longitudinally down the side. This may be reduced to two keels, appearing on the lateral side, when the cell is less active. All keels may not persist to the posterior end. Distinct striae parallel the twist of the keels. Small, neutral red-staining granules are scattered in the cytoplasm. The chromatophores are small, very numerous, disc-shaped, light yellowish-green and lie almost peripherally. They are close together and give the euglena the appearance of an almost solid color. No visible pyrenoids. The most noticeable paramylum is in the form of two long rods, one of which is posterior, the other anterior, of the centrally placed nucleus. Other arrangements than those mentioned are also known but are rare. There are also short rectangular blocks of paramylum. The flagellum is approximately ⅙ body length. The eyespot is bright red. This euglena swims with a rapid and rotating motion but frequently comes to rest, at which times it may be bent slightly into a "C" or "S." It is only slightly metabolic. Catarobic to mesosaprobic.

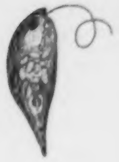

Fig. 61 *Euglena viridis*

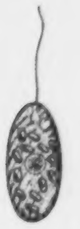

Fig. 62 *Astasia inflata*

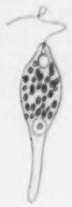

Fig. 63 *Astasia klebsii*

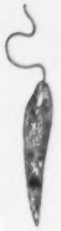

Fig. 64 *Astasia dangeardii*

*E. viridis* Ehrenberg: (Fig. 61) Size, 35 to 90$\mu$ by 10 to 22$\mu$. A fat spindle-shape but sometimes tending towards cylindrical. The anterior is rounded and the tail tapers to a point. The taper usually begins just posterior of the midpoint. The spiral striae are probably not recognizable without oil immersion. Neutral red-staining, spherical granules are arranged in four rows which may be irregular and which spiral to the right. Chromatophores, 7 to 12, are long, spearhead-shaped bodies in a star-shaped arrangement with the center of the star anterior of the nucleus. Pyrenoid occurrence is questionable. The paramylum bodies, in the shape of small ovals and bricks, are clustered around the chromatophoral star center. They are, however, also found in other parts of the cytoplasm. The active flagellum is longer than the body and is easily discarded or lost. The eyespot is crimson. The nucleus is post median and is partly obscured by the chromatophore bands. A rounded cyst without dense wall is formed. Metabolic, but not strongly so, and swims quite rapidly.

*Genus Astasia*: These strongly resemble the euglenas, but lack chromatophores and are therefore colorless. They also lack an eyespot. All are actively metabolic but in species which swim actively this may not be a noticeable characteristic. They are usually striated but striae may be seen in some only with oil immersion. Their classification is poorly defined.

*A. inflata* Dujardin: (Fig. 62) Size, 35 to 46μ. Cells flattened, pyriform (Pascher's illustration is nearly elliptical). Pellicular membrane is strongly helically striated. Flagellum is body length, and the nucleus is central. The paramylum granules are in the form of rods. Mesosaprobic.

*A. klebsii* Lemmermann: (Fig. 63) Size, 50 to 60μ by 13 to 20μ. Cells are spindleform with the posterior drawn out into an almost rodlike tail. Pellicular membrane indistinctly helically striated. The flagellum is about body length. The nucleus is located at the approximate midpoint. Paramylum granules have an oval shape. Meso-to poly-saprobic.

*A. dangeardii* Lemmerman: (Fig. 64) Size, 30 to 60μ by 12 to 20μ. Shape is inverted pyriform or half spindleform. The pellicular membrane is distinctly helically striated. Flagellum about body length. Nucleus posterior. Paramylum granules are oval. Cyst known, oval, with thin membrane. Mesosaprobic.

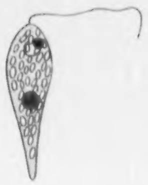

Fig. 65 *Astasia quartana*

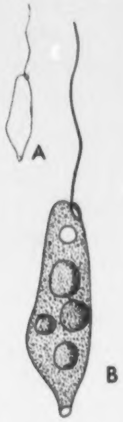

Fig. 66 *Euglenopsis vorax*
a. Outline
b. Detail

*A. quartana* (*Euglena quartana*) Moroff: (Fig. 65) Godjics has recommended that this organism be assigned to *Astasia* since it is not known to possess chromatophores. If an eyespot is present, it might more properly be assigned to *Khawkinea*. (Size, 50 by 15μ.) Spindle-form cells with posterior end reduced slightly, tail-like. Actively metabolic. Membrane smooth. Flagellum single, 1 to 1½ times body length. Paramylum granules most usually oval.

The above is Pascher's description (13) and his illustration indicates the presence of many scattered ellipsoid paramylum bodies, a typically located euglenoid eyespot, and a nucleus located in the posterior half. Since there is some doubt about the presence of eyespots in *Astasia*, it appears that *A. quartana* (or *Euglena quartana*) has served as a "catch-all" for euglenas which have lost their chromato-

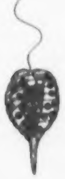

Fig. 67 *Phacus pyrum*

phores due to environmental or other conditions. Whether the species *quartana* is a valid species has not been investigated thoroughly and for that reason the species is included here until further evidence becomes available.

*Genus Euglenopsis*: This genus is differentiated from *Astasia* only by holozoic instead of saprophytic nutrition. Practically, the more lateral insertion of the flagellum and the difficult-to-discern mouth opening aid in differentiating. Food is taken in by the action of the mouth border. The residues of food may often be observed in an outpocketing of the posterior end. Very metabolic but also free swimming with a rotating motion.

*E. vorax* Klebs: (Fig. 66 a,b) Size, 20 to 30$\mu$ by 7 to 10$\mu$. Cells spindleform with a striated membrane (this may not be observable without oil immersion). Flagellum over body length. Cysts formed, spherical or oval, with a thick membrane. Poly- to meso-saprobic.

*Genus Phacus*: Cells of this genus are more rigid than those of the previous genera. They are slightly to distinctly flattened. Some are planoconvex and may or may not have keels, ridges, or other thickenings in the area of the longitudinal axis. Many have the lateral edges thinned down to winglike structures. One species possesses a dense shell on each broad side of the cell. Most possess chromatophores, but some are colorless. Pyrenoids may be present. They have gullet-reservoirs typically like those of the euglenas. Cells are single, free swimming and usually flattened with a firm, usually striated, pellicular membrane. The contractile vacuole and gullet-reservoir systems are similar to those in euglena. All have an orange-red eyespot near the base of the single flagellum. Chromatophores are numerous, disc-shaped with smooth or scalloped edges and lie adjacent to the pellicle. Paramylum granules variously formed, sometimes absent.

*P. pyrum* (Ehrenberg) Stein: (Fig. 67) Size, 30 to 55$\mu$ by 13 to 15$\mu$. Cell inverted pyriform with a drawn-out, pointed posterior which is circular in cross section. The body is almost circular in cross section and the helical striations are keel-like and give the body a twisted appearance. Flagellum is body length. It has two large, or many small, disc-shaped paramylum bodies. Oligosaprobic to catarobic.

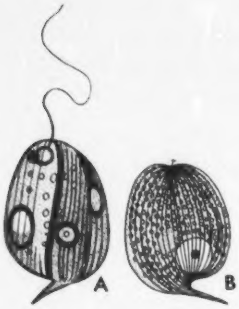

Fig. 68 a. *Phacus pleuronectes*
(Usual variety)
b. Phacus triqueter

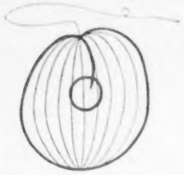

Fig. 69 *Phacus stokesii*

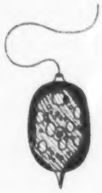

Fig. 70 *Lepocinclis ovum*

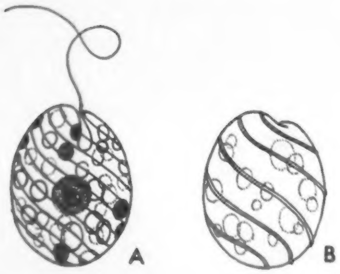

Fig. 71 *Lepocinclis texta*
a. } Showing differences
b. } in striation

*P. pleuronectes* (O. F. Muller) Dujardin: (Fig. 68 a,b) Size, 45 to 55$\mu$ by 30 to 35$\mu$. Possibly should include *P. triqueter* which differs in size and in having a dorsal fold extending to the posterior (instead of to the middle as will *P. pleuronectes*). These differences are not great enough to make separate species since intermediate forms can be found. Cells somewhat twisted and broadly oval in shape. They have a short, pointed, somewhat oblique-terminal spine. Flagellum is about body length or slightly longer. Paramylum present in the form of one or two ring-form bodies. Oligosaprobic to catarobic.

*P. stokesii* Lemmermann: (Fig. 69) Approximately 20$\mu$ (Conrad) to 46$\mu$ (Pascher) long. Cells broad, oval or almost round. Dorsal fold reaches almost to posterior end. Membrane longitudinally striated. Flagellum body length. One round paramylum body posteriorly located.

*Genus Lepocinclis* (Crumenula of Kudo): Body rigid, usually circular in cross section. An anterior papillary projection from which the flagellum arises may be present. Characteristic large, ring-form paramylum bodies are lateral. Lepocinclids are typically euglenoid in striation, gullet-reservoir, vacuole system and other structures. Green cells with numerous chromatophores, which are usually discoid and peripherally located, without visible pyrenoids. Colorless lepocinclids have not been reported from pollution surveys.

*L. ovum* (Ehrenberg) Lemmermann: (Fig. 70) Size, 25 to 40$\mu$ by 15 to 18$\mu$. Cells elliptical with a distinctly "set on" posterior spine (6 to 7$\mu$ long). Membrane clearly helically striated. Flagellum double body length. (Pascher (13) lists several other varieties of this species whose sizes begin at 20$\mu$ by 15$\mu$ and which show variation in morphology, pellicle markings and flagellum length.)

*L. texta* (Dujardin) Lemmermann: (Fig. 71 a,b) Size, 52 to 60$\mu$ by about 38$\mu$. Cells broadly elliptical. Membrane distinctly helically striated. Flagellum two to three times body length. Paramylum bodies numerous, cylindrical, spherical or ring-shaped. Meso- to oligo-saprobic.

*Genus Trachelomonas*: These euglenoids inhabit a spherical, elliptical, cylindrical or occasionally oval or otherwise shaped shell or other armored covering known as a lorica or test. Such

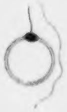

Fig. 72 *Trachelomonas volvocina*

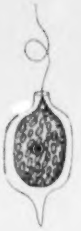

Fig. 73 *Strombomonas (Trachelomonas) urceolata*

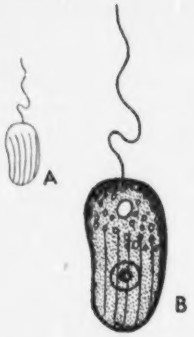

Fig. 74 *Rhabdomonas incurva*
a. Outline
b. Detail

tests are composed of cellulose, pectin, tectin, or other substances. The test completely surrounds the protoplast except for a pore through which the single flagellum extends. The flagellar pore is usually thickened or surrounded by a collar which often has a distinctly "set on" appearance. If old, the loricae are frequently discolored with iron or manganese, and such coloring is often so heavy that the details of the organism cannot be distinguished. The organisms within the shells have chloroplasts, eyespots, gullet-reservoirs and, usually, paramylum like the naked euglenas. When discernible, the protoplast seems entirely free in the lorica; it is strongly metabolic and the chromatophores are discoid or bowl-shaped, with or without pyrenoids. If the lorica is crushed, the freed organism cannot be distinguished from the euglenas. A few species lack chromatophores. Differentiation of species is usually on the basis of shape and ornamentation of the lorica. Huber-Pestalozzi (6) lists 233 species and many varieties of *Trachelomonas,* with 42 species of *Strombomonas* plus several varieties. They are free swimming. Representatives have been reported from polluted streams. They are not found in treatment units.

*T. volvocina* Ehrenberg: (Fig. 72) Lorica 5 to 25μ in diameter, spherical and not ornamented. The flagellar pore has a thickened ring or, rarely, a low collar. The shell is relatively dense. Flagellum 2 to 3 times body length. The chromatophores have pyrenoids. Pascher lists three varieties, differentiated on the basis of a short collar extending inward, slightly elongated lorica and flagellar pore with a collar arising from a papillalike structure.

*Genus Strombomonas*: This genus has been recently erected to include organisms formerly classified as *Trachelomonas* but whose loricae are pliable and have necks which are continuous with the bodies of the loricae. Commonly a single posterior point characterizes members of this genus and it is usually continuous with the body of the lorica (i.e., it does not look "set on").

*S. urceolata* Stokes (often listed as *Trachelomonas urceolata)*: (Fig. 73) Length, 20 to 60μ. The body of the lorica is somewhat cylindrical with a necklike anterior which is not bent and whose plane of opening is perpendicular to the long axis. Posterior somewhat spinelike. Flagellum approximately body length.

*Genus Rhabdomonas*: Members of this genus have rigid, curved bodies. They are colorless. Flagellum single from a typical gullet-reservoir. Paramylum is usually visible. Motion rapidly free swimming with rotation. This genus recently has been separated from *Menoidium* because of a rounded cross section. The body shows distinct longitudinal ridges about evenly spaced.

*R. incurva* Fres. (*Menoidium incurvum*): (Fig. 74 a,b) Size, 15 to 25μ by 7 to 8μ. Cells slightly bent, cylindrical, with both ends broadly rounded. Striated by distinct ridges which flatten out posteriorly. Flagellum approximately body length. Paramylum as discs. Nucleus posterior to central. Cysts form directly from active cells but anterior is sometimes truncated. Mesosaprobic.

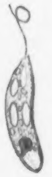

Fig. 75 *Menoidium pellucidum*

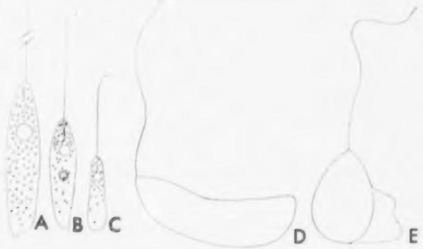

Fig. 76 *Peranema trichophorum*
a. Outline
b. Detail
c. A smaller organism, showing different morphology
d. Outline of an organism beginning to turn
e. Organism further turned showing folding of body

*Genus Menoidium*: Similar to *Rhabdomonas* but with cells compressed and curved spindle-shaped, or at least asymmetrical in side view. Some exhibit twisting as well as curving. Pellicle smooth or very faintly striated. Anterior end may flare slightly from a more or less narrow neck. The "flare" is transversely truncated. Paramylum, generally rods which are frequently short. Gullet-reservoir usually conspicuous.

> *M. pellucidum* Perty: (Fig. 75) Size, approximately 40$\mu$ by 7 to 10$\mu$. Cells slightly bent with reduced, rounded posterior end. Anterior with the neck and flare typical of the genus. Membrane finely striated. Flagellum somewhat over ½ body length. Nucleus posterior. Paramylum cylindrical or rectangular rods.

**Suborder Peranemoidina:** Colorless cells, very metabolic with one or two flagella but sometimes, even when two are present, only one may be readily visible. If two flagella are present, they are unequal and the smaller flagellum usually trails. However, in some it may be active and extend in a latero-anterior direction. One or both flagella are thickened near the base (this thickening—probably a protoplasmic sheath—may extend over much of the flagella length); hence, the ready visibility of the flagella of *Peranema* and others. Pellicle with delicate striation, usually visible only with oil immersion. Certain families of the Peranemoidina have rodlike bodies lying beside the neck of the gullet-reservoir, near the anterior end. These rod organs are quite characteristic and can ordinarily be found either in living or stained organisms. Both holozoic and saprophytic nutrition occur; both may possibly occur in the same individual.

*Genus Peranema*: Strongly metabolic cells having rod organs and one or two flagella with the anterior portion of the principal flagellum usually the active portion. Cells elongately pyriform, elongately oval or posteriorly truncate spindleform with two distinct, but not always readily observable, nail-like rod organs. Pellicle very finely striated or granular. Flagella two, but only one readily observable. Principal flagellum carried stiffly forward with the anterior tip vibratile. Paramylum present.

*P. trichophorum* (Ehrenberg): (Fig. 76 a,b,c,d,e) Size, 20 to 110μ by 12 to 20μ. Highly metabolic. It has been rather aptly described as a laundry bag dragged around by its string. The string in this case is the swimming flagellum which is one of the heaviest encountered in the entire flagellate group. It is carried stiffly forward and only the anterior ⅓ to ¼ vibrates. When turning, however, or resting in one place, the entire flagellum may show a lashing wavelike motion. Under oil immersion, the pellicle shows fine striae. The rod organs are fairly heavy and often set at a slight angle to the long axis. The anterior end is roundly pointed and the principal flagellum appears to emerge from its tip. The posterior is truncated, usually obliquely and irregularly. The body might be termed a roundly and irregularly truncated spindle. While this organism is frequently classified as monoflagellated, an adhering secondary flagellum does exist (10). The gullet-reservoir and the adjacent contractile vacuole are difficult to observe clearly. This species engulfs other organisms by expanding the gullet and "swallowing the food" in a number of expanding and contracting movements. Sandon (16) states that their food consists of other euglenoids, but in sewage they have been observed consuming many other flagellates and may possibly eat bacteria at times. Paramylum granules in the form of small discs frequently obscure the internal structure of these organisms when found in sewage. The nucleus is approximately central. The organism glides rapidly straight ahead. It often folds on itself and changes direction, or may change without folding.

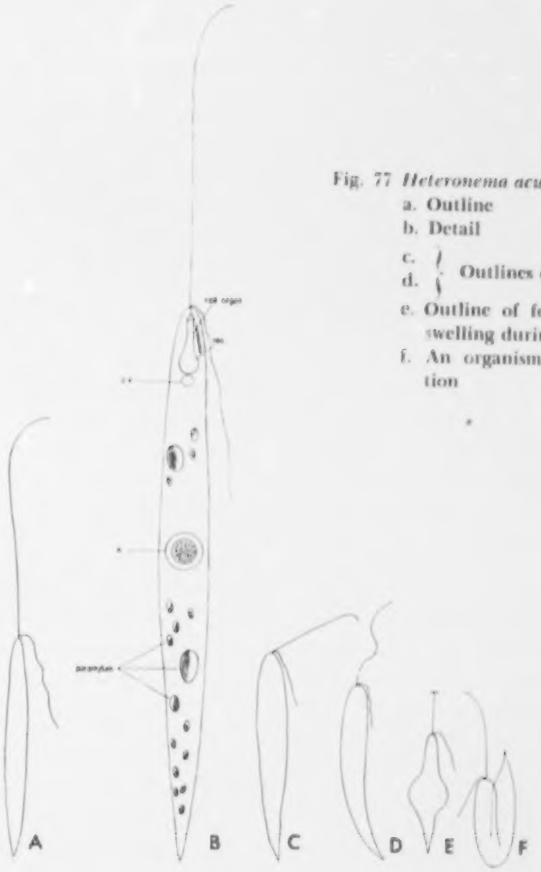
rod organ
paramylum
A
B
C
D
E
F

Fig. 77 *Heteronema acus*
a. Outline
b. Detail
c. }
d. } Outlines of swimming forms
e. Outline of forms showing medial swelling during metaboly
f. An organism reversing its direction

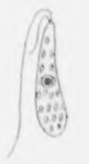

Fig. 78 *Heteronema tremulum*

*Genus Heteronema*: Strongly metabolic cells which when fully extended are cylindrically spindle-shaped or elongately oval. Some have elevated spiral ridges, others have a smooth surface on which dense spiral striae may be observed with oil immersion. Flagella two; the swimming flagellum the longer, the trailing flagellum rarely as long as the body. Usually the swimming flagellum is carried stiffly forward with only the distal 1/4 to 1/5 vibratile. A pharyngeal rod apparatus (rod organ) is present but is more delicate than in *Peranema*.

*H. acus* (Ehrenberg) Stein: (Fig. 77 a,b,c,d,e,f) Size, 40 to 100$\mu$ by 6 to 20$\mu$. The organism generally described as this species in this country does not fit Pascher's description (13) which he says has both ends rounded. (Pringsheim disagrees with Pascher and states that the two conform.*) American forms are described as having a pointed posterior formed as the end of an elongated taper. Since size and other characteristics agree rather well, the variety name *americanus* is suggested for the organism found in this country. Cells elongated, spindleform or almost fusiform with rounded anterior, pointed posterior, and very fine spiral striae which may be seen readily only with oil immersion. Cells are very metabolic, the central portion may enlarge to three or four times its normal diameter. Swimming flagellum somewhat over body length, trailing flagellum up to ½ body length. Rod organs similar but somewhat more delicate than those of *Peranema trichophorum*. Nucleus central. Movement gliding, usually with metaboly. Paramylum, small round granules.

*H. tremulum*: (Fig. 78) Size, 40$\mu$. Cells elongately oval and curved or, as Pascher (13) describes it, "weakly sickleform, anterior smaller, posterior broadly rounded out." Membrane smooth. Swimming flagellum somewhat over body length, trailing flagellum ¼ body length. So far not reported from this country.

*Genus Distigma*: Strongly metabolic, colorless flagellates having two unequal flagella. Body shape when organism is quiescent more or less spindleform. Flagella may or may not be equally active. They are rarely free swimming in slide preparations. Note: Pringsheim classifies *Distigma* as an *Astasia* because of (1) its nutrition, and (2) the type of metaboly.*

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*Verbal communication.

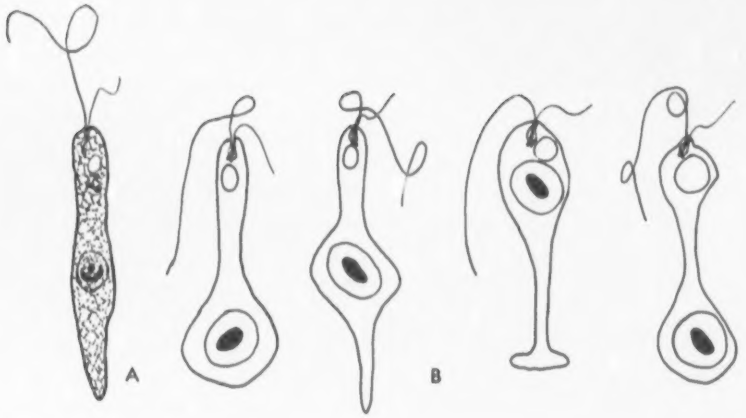


Fig. 79 *Distigma proteus*
a. Swimming cell
b. Showing the progressive changes in shape during active metaboly (not to size)

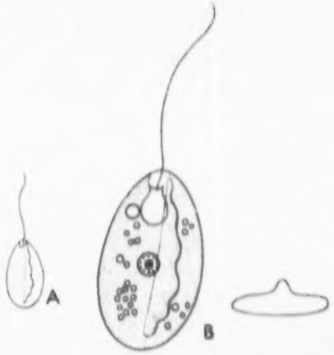


Fig. 80 *Petalomonas carinata*
a. Outline
b. Detail with cross section

*Distigma* is left here in spite of Pringsheim's excellent reasons because (1) it has the heavy (sheathed) flagella, and (2) Lackey reported two large and distinct granules near the bulbous portion of the gullet-reservoir (rudimentary rod organs?).

*D. proteus* Ehr: (Fig. 79 a,b) Size, 45 to 110$\mu$. Cells, highly metabolic, exhibit a great variety of shapes including a quite characteristic dumb-bell shape not seen in other groups. The shape, when the organism is not actively moving, tends to be somewhat spindleform. The pellicle is very finely striated, but the striation can be seen readily only with oil immersion. The two flagella are quite different in length and seem to act somewhat independently; thus, the longer primary flagellum may be directed stiffly forward while the shorter secondary flagellum exhibits a wavy motion at a distinctly oblique angle to the axis of the animal. Many variations may be seen in the pattern of flagellar action, even to having, rarely, the longer flagellum trailing. Paramylum granules are cylindrical. Nucleus variously located due to metabolic action but approximately central in spindleform cells. This organism has a striking and characteristic metaboly. Nutrition appears to be saprophytic.

**Suborder Petalomonadoidina:** More or less rigid and frequently compressed euglenoid flagellates. They possess one or two flagella, and each flagellum is thickened (sheathed?) at the base. A few have rod organs.

*Genus Petalomonas*: Flattened, somewhat rigid cells. They are generally pyriform, oval or rectangular in outline but posteriorly may show points, lobes, or other structures. Almost all exhibit longitudinal keels, grooves, channels, or similar structures. Some are distinctly asymmetrical. The single flagellum usually carried forward with motion in the distal end. Contractile vacuole anterior, generally on the left. Nucleus usually on the right at the midpoint. Lacking rod organs. Creeps or glides, often with a quivering motion.

*P. carinata* France: (Fig. 80 a,b) Size, approximately 23$\mu$. Cells long, pyriform with rounded ends. Keel not greatly raised and inrolled ventrally from the right. Contractile vacuole and nucleus behind one another in the midline. Lackey (9) tentatively assigned to this species an organism which had a lobed border on the keel.

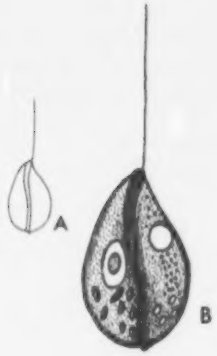


**Fig. 81** ***Petalomonas mediocanellata***
**a. Outline**
**b. Detail**

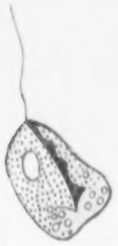


**Fig. 82** ***Petalomonas steinii***

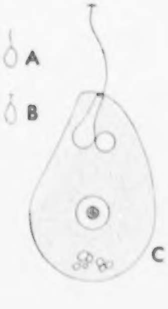


**Fig. 83** ***Scytomonas pusilla***
**a. Outline**
**b. Outline of a slightly different form**
**c. Detail**

*P. mediocanellata* Stein: (Fig. 81 a,b) Size, 22 to 25$\mu$. Cells broad pyriform and flattened. Both ventral and dorsal sides furrowed. Dorsal furrow smaller. Left border of ventral furrow stands out more sharply. Flagellum single, approximately body length. Paramylum as small discs or spheres.

*P. steinii* Klebs: (Fig. 82) Size, 38 to 42μ by approximately 22μ. Cell pyriform, in transverse section three edged. With a distinct longitudinal keel and sharp lateral borders. Flagellum single, body length, or somewhat longer.

*Genus Scytomonas*: Cells rigid, oval to elliptical without pellicle markings. Glides over the substrate with a peculiar flopping action of the flagellum. The single flagellum is one of the heaviest flagella among the flagellates in proportion to the size of the organism. *Copromonas* is considered to be a synonym.

*S. pusilla* Stein: (Fig. 83 a,b,c) Size, 10 to 15μ (less frequently 7.5 or up to 20μ) (Pascher); 5 to 10μ (Calaway). The many *S. pusilla* encountered from various secondary sewage treatment processes only rarely exceeded 7 to 8μ in size, the most common size being about 6.5μ. Cells ovate with the anterior portion displaced laterally and the body length flagellum originating a short distance from the anterior tip on the more convex edge. The organism is compressed and rests about as frequently on one side as on the other. Under the oil immersion, the flagellum is seen to spring from a gullet-reservoir system. The peculiar action of the flagellum is such that the anterior half is most active but the whole flagellum takes part in and describes a funnel-like pattern. The flagellum frequently acts for a few seconds and then rests for an even shorter interval. The action is mostly confined to about 30 to 40 degrees on each side of an extension of the primary axis. Motion is straight forward for a short interval and then at an angle in another direction. The body appears to be in contact with the substrate at the anterior end, while the posterior swings freely. The flagellum may, however, act perpendicularly to the body, almost turning the organism about. Nucleus is approximately at the midpoint and visible with oil immersion. Cysts formed. Zygotes have been reported.

*Genus Notosolenus*: Cells of this genus are oval to almost bell-shaped in outline, rigid and distinctly flattened. The dorsal side is concave, the ventral side usually convex. Only one flagellum may ordinarily be seen and, when the organism is moving steadily, it is carried stiffly and always obliquely forward, with the anterior 1/3 to 1/5 vibrating. It is about 1⅛ times body length. A secondary flagellum is present but it is most

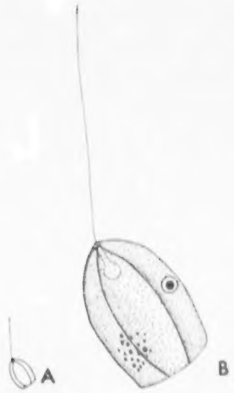


Fig. 84 *Notosolenus apocamptus*
a. Outline
b. Detail

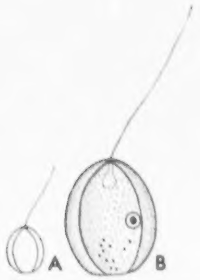


Fig. 85 *Notosolenus orbicularis*
a. Outline
b. Detail

difficult to see. It is usually about ⅓ to ½ body length and carried trailing, close to the ventral side. Pascher states that these organisms are furrowed, but Lackey (9) suggests that, at least in the species *orbicularis*, instead of a furrow, a broad depression is present which gives the entire body a concave-convex shape. Observations indicate that the depression in some species is elongated and extends to the posterior margin, sometimes producing a slight depression of that margin.

*N. apocamptus* Stokes: (Fig. 84 a,b) Size, 6.5 to 10.5$\mu$ (Pascher). A number of organisms believed to belong to this species were 15 to 17$\mu$ long, oval and somewhat asymmetrical. The rigid body is almost rounded in outline, distinctly flattened and with a broad shallow depression slightly back of the flagellar insertion, making the body concave-convex. Two flagella are present but the trailing one is very difficult to observe. The swimming flagellum is approximately 1½ body length, and the trailing flagellum, ⅓ body length. The cytoplasm is clear except for a few granules, and the pellicle is smooth. The flagellate moves steadily with the flagellum carried obliquely, its anterior portion vibrating while the organism glides over the substrate. It also swims freely with a shallow wavelike action of the flagellum and vibration of the body. The organism here described lacks the pointed anterior of the organism described by Pascher (13) and also lacks the seams along the borders of the dorsal depression. Pascher's statement that a primary and a secondary vacuole exist has not been confirmed by personal observation.

*N. orbicularis* Stokes: (Fig. 85 a,b) Size, 10 to 17$\mu$ long. These cells are definitely oval but somewhat asymmetrical in outline, with a slightly rounded ventral side and a depressed dorsal side. The dorsal depression is elongated and may extend to the posterior margin as a very shallow broad groove. Two flagella are present but the trailing one is only ⅓ to ½ body length and is rarely observed. The obliquely carried swimming flagellum is about body length, only its anterior portion vibrating in steady forward motion. When not moving steadily, the flagellum frequently shows a wavelike action in a lateral direction, and the organism may rotate using a point on the body as a pivot and also shift position. It frequently tips obliquely on the left anterior border and glides along in a wobbly manner in this position. The gullet is in the left margin. Pascher (13) states that a primary and a secondary vacuole exist. Light refractive bodies, presumably paramylum, are scattered through the body. These are especially noticeable in border areas. In some individuals the right border is somewhat narrower than the left, and its inner edge tends to overhang slightly the central depression.

*Genus Anisonema*: Cells metabolic or rigid, oval or elongately oval in outline and dorso-ventrally compressed. Ventral side somewhat flattened but possessing a groove in which the trailing flagellum lies. Two flagella spring from the gullet which opens ventrally somewhat back of the anterior edge. In all members of the genus, except *A. emarginata*, the swimming flagellum is about body length. A generic characteristic is the thickness of the basal portion of the trailing flagellum, which may lie freely in the ventral groove. It is always longer than the swimming flagellum. A gullet-reservoir and associated contractile vacuole may frequently be distinguished. A siphon similar to that of *Entosiphon* has been reported but its existence is questionable at best.

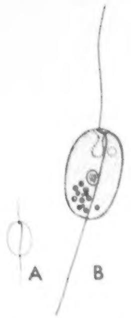


Fig. 86 *Anisonema ovale*
a. Outline
b. Detail

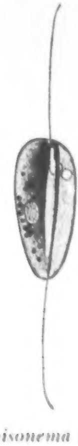


Fig. 87 *Anisonema truncatum*

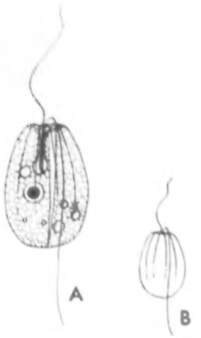


Fig. 88 *Entosiphon sulcatum*
a. Outline
b. Detail

*A. ovale* Klebs: (Fig. 86 a,b) Size, 11$\mu$ by 7$\mu$. Cells rigid, oval, with a rounded anterior. Pellicle smooth. Swimming flagellum body length; trailing flagellum 1½ times as long. Catarobic to oligosaprobic.

*A. emarginatum* Stokes: Size 14$\mu$. Cells rigid, broadly oval with rounded anterior. Pellicle smooth. Swimming flagellum approximately double body length, trailing flagellum a little longer.

The writers believe that *A. emarginatum* and *A. ovale* are not distinct species. *A. ovale* was the first described.

Only the illustration of *A. ovale* is shown (Fig. 86 a,b).

*A. truncatum* Stein: (Fig. 87) Size, 60μ by 20μ. Cells rigid, inverted pyriform. Pellicle smooth. Swimming flagellum about body length, trailing flagellum more than twice as long.

*Genus Entosiphon*: Cells oval to elliptical in outline with a rounded truncation of the anterior end, not strongly flattened, fairly rigid, circular in cross section or only slightly compressed. Generally larger than 20μ long. The pellicle is dense and has longitudinal ribs which produce furrows at the anterior end. A relatively large bulb with a short, narrow neck forms the gullet-reservoir. All members of this genus have a distinct siphon located in or near the longitudinal axis of the cell and which reaches ¾ or more of the distance to the posterior margin. This apparatus is similar to, and may be homologous with, the rod apparatus of *Peranema*. The organism glides on the substrate with a trembling motion. The trailing flagellum, while usually inactive, appears to snatch the organism out of places where it tends to become wedged. Smith (18) states *E. ovata* is probably a variety of *E. sulcatum*, but they will be here described as separate species.

*E. sulcatum* (Dujardin) Stein: (Fig. 88 a,b) Size, 20 to 30μ by 10 to 22μ. Cells elliptical with 4 to 8 longitudinal ribs which are most noticeable at the anterior end. Swimming flagellum approximately body length, trailing flagellum a little longer. A conical siphon reaches to the posterior border. It sometimes protrudes slightly and may produce a squared-off rostrum on the central part of the anterior margin. Cell is usually noticeably compressed and thus has an elliptical cross section but almost circular transverse sections may frequently be seen. The swimming flagellum is carried forward usually as a fairly tight curve (forming a "U"-like shape) and the entire flagellum vibrates in swimming. The trailing flagellum, though normally inactive, does at times jerk the organism backwards. Oil and paramylum both may be present as inclusions. The reservoir is large and easily seen, and the contractile vacuole is attached or adjacent to its side. The nucleus is at one side in the equatorial region. Moves intermittently on the substrate with a trembling motion, seldom swims freely.

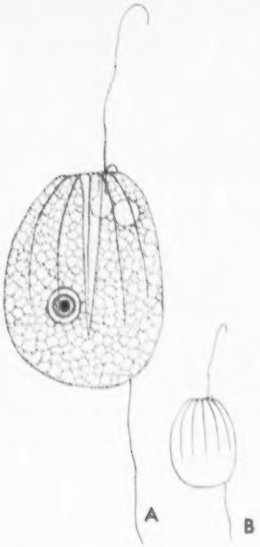


Fig. 89 *Entosiphon ovatum*
a. Outline
b. Detail

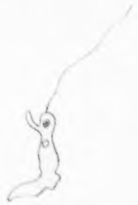


Fig. 90 *Mastigamoeba longifilum*

*E. ovatum* Stokes: (Fig. 89 a,b) Size, 25 to 28μ. Somewhat more ovate than the previously described species. The siphon does not quite reach the posterior margin, and the secondary flagellum is 1½ times body length or slightly more. Longitudinal striae number 10 to 12. The organism otherwise conforms to the description of *E. sulcatum*.

## CLASS ZOOMASTIGOPHOREA

Some of the characteristics of this class have already been mentioned (page 19). The group consists of organisms which, in most cases, have highly plastic bodies. Feeding is often in an amoeboid manner although saprophytic (saprozoic) nutrition is believed to be fairly common. Some members of this group may be transferred to the *Phytomastigophorea* when knowledge of their characteristics becomes more complete.

**Order Rhizomastigida:** Flagellates with 1 to 4 flagella (but only 1, or possibly 2, flagella in known sewage forms) and with highly amoeboid bodies. Because of this amoeboid characteristic, they may be erroneously referred to the subphylum *Sarcodina* by workers without experience.

FAMILY MASTIGAMOEBIDAE: In this group, known sewage forms have one flagellum only. All produce pseudopodia which show considerable variation from species to species. Because of their amoeboid characteristics, body form can be described only in very general terms, and the body does not return to a fairly typical morphology as is true with most flagellates. These flagellates are relatively small and, since they frequently adhere to the substrate, even when their flagellum is active, they are easily overlooked. The flagellum is long (2 to 3 times body length or greater) and fairly slender; it is usually very active.

*Genus Mastigamoeba*: In this group, the flagellum and its basal granule are attached by means of a rhizoplast or axial fibril to the nucleus. The nucleus is located in the anterior half of the cell. The fibril is usually readily distinguished but must be carefully searched for in some cases. The cells show the amoeboid body and the elongated fine flagella of the family. Partial descriptions of several members of this group have been made (9) and it is apparent that some undescribed species exist. Two or three species are fairly common.

*M. longifilum* Stokes: (Fig. 90) Size, 20 to 35$\mu$. This form swims freely or creeps on the substrate. The cells are usually long and narrow, and numerous short pseudopodia arise from any point on the body surface. The flagellum is twice the length of the extended animal or slightly longer. The flagellum is vibratile near the tip when swimming and points directly anteriorly. When the organism is on the substrate, it may extend in any direction. The attachment of the flagellum to the nucleus is difficult to see. The nucleus is placed well forward. A single contractile vacuole is adjacent to the nucleus.

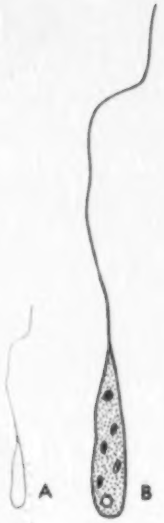


Fig. 91 *Mastigamoeba limax*
a. Outline
b. Detail

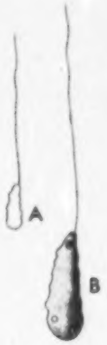


Fig. 92 *Mastigamoeba reptans*
a. Outline
b. Detail

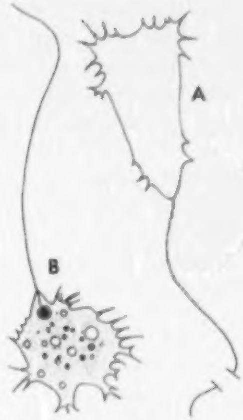


Fig. 93 *Mastigamoeba sp. I*
a. Outline
b. Detail of cell with somewhat different morphology

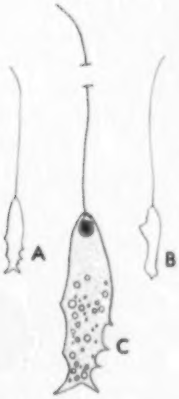


Fig. 94 *Mastigamoeba sp. II*
a. Outline
b. Outline of the animal showing pseudopodia formation
c. Detail

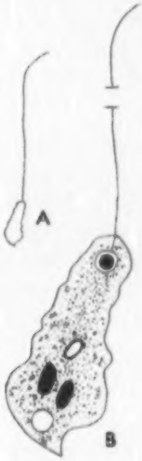


Fig. 95 *Mastigamoeba sp. III*
a. Outline
b. Detail

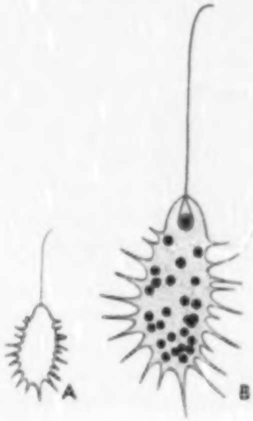


Fig. 96 *Mastigamoeba chlorelligera*
a. Outline
b. Detail

*M. limax* Moroff: (Fig. 91 a,b) Size, 20 to 25μ by 4 to 6μ. Cells elongated, pyriform. This species is frequently free swimming and lacks pseudopodia when swimming. The cuticular layer is smooth. Pseudopodia, short and broad. The flagellum is long, being 2 to 3 times the body length. Contractile vacuole present.

*M. reptans* Stokes: (Fig. 92 a,b) Size, 14μ. Cells pyriform with lobed, short pseudopodia. Flagellum 2 to 3 times body length. Possessing two or more contractile vacuoles, one of which is usually posterior.

*M. sp.* (Lackey) I: (Fig. 93 a,b) Size, 40 to 60μ. Close to *M. ramulosa* S. Kent. Body ovate to elongate bearing numerous short and sharp pseudopodia. Flagellum very long (up to 120μ) arising from the nucleus which is constant in location. There are numerous small vacuoles; this is the point of greatest difference from *M. ramulosa*. Endoplasm with many small granules.

*M. sp.* (Lackey) II: (Fig. 94 a,b,c) Size, 25 to 35μ Irregular in shape with short blunt pseudopodia that arise from any portion of the body. Flagellum 2 to 3 times body length. Swims rapidly, waving the tip of the flagellum. Easily recognized because it has no contractile vacuole. Small refringent granules are enclosed in the posterior portion of the body.

*M. sp.* (Lackey) III: (Fig. 95 a,b) Size 15μ or less; rarely larger. Few or no pseudopodia formed, but cell very amoeboid. Anterior part hyaline, a few foreign bodies in the posterior. One posterior contractile vacuole. Flagellum quite long (60μ) and vibratile at the tip.

*M. chlorelligera* (*M. viridis* Lackey) new name: (Fig. 96 a,b) The above change in name is suggested for the organism reported as nov. sp. in 1925 (9). Pascher (12), in a foot note, mentioned *M. viridis* described by Prowazek. Since the organisms, though in many respects similar, have some distinct differences, it seems wise to assume prior usage of the name, *viridis,* and rename the organism described by Lackey as indicated.

Size, 25 to 30μ by 12 to 20μ. Cells somewhat oval in shape. The body does not exhibit a great change in form. Long pointed pseudopodia arise over the entire surface.

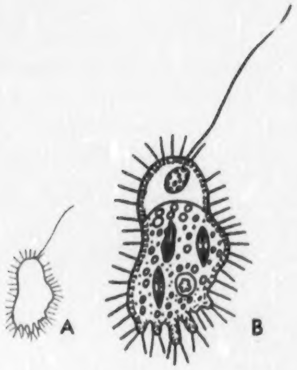


Fig. 97 *Mastigamoeba trichophora*
a. Outline
b. Detail

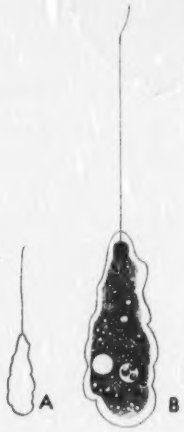


Fig. 98 *Mastigamoeba volutans*
a. Outline
b. Detail

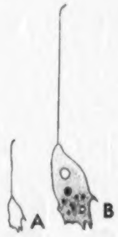


Fig. 99 *Mastigella simplex*
a. Outline
b. Detail

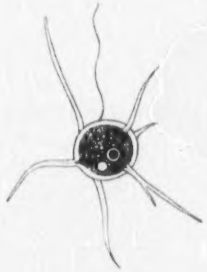


Fig. 100 *Mastigella radiosa*

They somewhat resemble the pseudopodia of the helizoans. Flagellum about twice body length, arising from a nipple-shaped projection. Nucleus easily visible, located in this nipple. Protoplasm absolutely clear, hyaline and devoid of any foreign particles except a number of chloroleucites. These chloroleucites are not bright green, but are of a greenish yellow color, suggesting that they contain xanthophyll rather than chlorophyll. Contractile vacuoles have not been seen. Found in Imhoff tank digestion chamber.

*M. trichophora* Lauterborn: (Fig. 97 a,b) Size, about $100\mu$ by 30 to $45\mu$. Cells elongated with a distinct alveolar ectoplasmic layer of uniform thickness and having a covering of many fine bristles. Flagellum approximately ⅔ body length. Contractile vacuoles absent. With ectoplasmic granules.

*M. volutans* Lackey: (Fig. 98 a,b) Size, 25 to 30μ. Body elongated, flattened. Flagellum vibratile in its anterior third, slightly tapering, 1⅓ to 2 times body length. Cortical layer of protoplasm clear, hyaline; endoplasmic portion finely granular, reticulate with numerous small, square crystals and numerous spheres of varying sizes. Due to the fact that these spheres stain with methylene blue and that, in some of these animals, a clear vacuole was found which contained from one to three of these spheres, they were assumed to be volutin. Pseudopodia very short, numerous, rounded. Contractile vacuole single, in posterior part of cell. Nucleus near anterior tip of cell, round in outline with a central endosome surrounded by minute chromatin granules within a clear zone of nuclear sap. Membrane thin. A rhizoplast visible between the nuclear membrane and the point of flagellar insertion. Nutrition apparently saprozoic.

*Genus Mastigella*: Cells free swimming or creeping amoebalike. Pellicle layer often with granules. Endoplasm frequently with rod-shaped crystals (bacteroids). Pseudopodia usually present, rarely entirely absent. One swimming flagellum with a basal granule and no visible connection to nucleus. Contractile vacuole, one to several. Nutrition holozoic or saprophytic.

*M. simplex* S. Kent: (Fig. 99 a,b) Size, approximately 12.5μ. Cells elongated and with a reduced posterior. Pseudopodia, confined almost entirely to the posterior end, small, sharp and sometimes drawn out long. Flagellum about double body length. Contractile vacuole not reported. Nucleus near the middle. Lackey (9) relates to this species an organism 25 by 12μ having two contractile vacuoles and with clear anterior and granular posterior cytoplasm.

*M. radiosa* Lackey: (Fig. 100) Size, about 20μ in diameter. Animal with a spherical body, free swimming or floating. Pseudopodia much like those of *Amoeba radiosa,* occasionally branching, clear, up to 80μ long. Single flagellum 100μ or about 5 times body length, used with a lashing or whiplike motion. One contractile vacuole. Nucleus central, spherical with endosome, membrane very thin, chromatin granules small. Cytoplasm finely granular, no zones being present. Several types of inclusions, such as oil spheres or crystalline bodies present. Nutrition saprozoic.

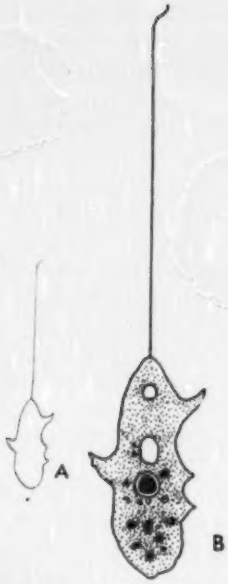


Fig. 101 *Mastigella sp. I*
a. Outline
b. Detail

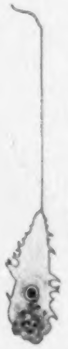


Fig. 102 *Mastigella sp. II*

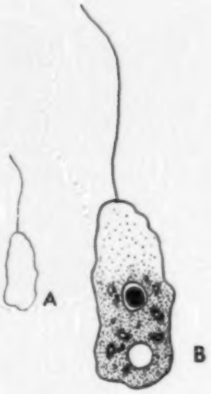


Fig. 103 *Mastigella sp. III*
a. Outline
b. Detail

*M. sp.* (Lackey) I: (Fig. 101 a,b) This is one of three described previously (9) but to which no species name was assigned (see also the two *Mastigella* sp. below). Size, 30μ long, 12μ wide. This species is similar to *M. simplex* in that it has short pointed pseudopodia. It differs from *M. simplex* in that the pseudopodia arise from any point on the body instead of being confined to the posterior end and in that the pseudopodia are somewhat more lobose than those of *M. simplex*.

*M. sp.* (Lackey) II: (Fig. 102) Size, 40 to 50μ. General contour elongate ovoid to elongate pyriform. The pesudopodia form a network on the margin of the organism (reticulate pseudopodia). The nucleus is central and vacuoles are lacking. The flagellum is three to four times the body length and vibratile near the end. The anterior cytoplasm is hyaline.

*M. sp.* (Lackey) III: (Fig. 103 a,b) Size, 25μ. This approximately ovoid organism is similar to *M. commutans* (H. Meyer) Goldschmidt, but lacks the five times body length flagellum. The flagellum in this species is slightly more than body length. The anterior cytoplasm is clear to slightly granular and the posterior more dense, containing foreign matter. Pseudopodia are not found but the whole body is somewhat amoeboid. One contractile vacuole, located posteriorly.

**Order Protomastigida:** These are mostly small forms with one or two flagella. Many show amoeboid tendencies, but these are not so marked as in the previous order (Rhizomastigida), and they return, in most cases, to a more or less definite body shape. Some are colonial, and some are solitary; some are armored, and some naked; some free swimming, and some attached. The life cycle of the forms found in sewage is simple, and sexual reproduction is probably not known. Many members of this group are found in sewage treatment processes and in polluted streams. Because of their small size and variable morphology, many members of the group are difficult to differentiate.

**Suborder Monomastigina,** suborder novum: Small monoflagellated organisms. Many have loricae, many others possess protoplasmic collars. Colony formation is common, but a few are solitary. Attachment, with or without stems, is common.

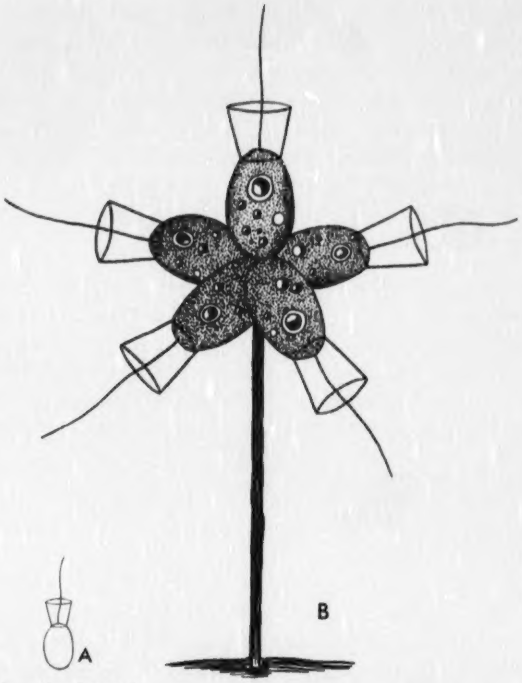


Fig. 104 *Codonosiga botrytis*
a. Outline of free swimming individual
b. Colony, showing detail

FAMILY CODONOSIGIDAE: Small monoflagellates with protoplasmic collar around the flagellum. Members of this family do not have loricae. Stemmed forms are frequent, and several are colonial.

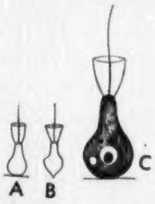


Fig. 105 *Monosiga ovata*
a. Outline
b. Outline of a cell with slightly different morphology
c. Detail

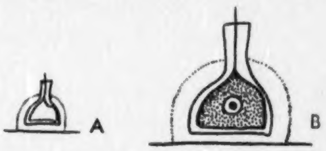


Fig. 106 *Salpingoeca marssonii*
a. Outline
b. Detail

Organisms swim with the flagellum backwards. Feed on bacteria, but may also be saprophytic. Colonial or solitary.

*Genus Codonosiga*: Cells as clusters on the ends of simple or branched stems. Collars very high. Stems long.

*C. botrytis* (Ehrenberg) S. Kent: (Fig. 104 a,b) Size, to 30$\mu$. Cells single or in groups on the ends of stems which are 2 to 10 times body length. Cells spherical or inverted pyriform. Collar height about ½ body length. Flagellum about 1½ times body length. Two varieties have been described.

*Genus Monosiga*: Cells solitary, sometimes stemmed, but usually with short stems when present. Sometimes with short pseudopodia.

*M. ovata* Kent: (Fig. 105 a,b,c) Size, 5 to 15$\mu$. Cells spherical to pyriform. Attached to the substrate either directly or by means of a short stem. Collar height approximately body length. Flagellum approximately twice body length.

FAMILY BIOCOECIDAE: Loricated, small, monoflagellated organisms with or without collars. Collars are sometimes very rudimentary and difficult to see. Hall (4) refers many members of this family to the previous family and indicates that others belong to the colorless chrysomonads, but does not transfer them. For this reason and because of the convenience of segregating loricated organisms, the classification used by Kudo (8) is retained here. The organisms of this family may be solitary or colonial, and some possess two collars.

*Genus Salpingoeca*: Cells living in, and attached to, the loricae, either directly or by means of a stem. Loricae usually attached to the substrate at the end opposite the flagellum, either directly or by means of a stem. Protoplasmic collar extending outside the lorica, infrequently contained within the lorica. Protoplasmic collar single. A single contractile vacuole. Cysts formed within loricae spherical to oval, with thin membrane or naked. Reproduction by longitudinal division, rarely by budding.

*S. marssonii* Lemmermann: (Fig. 106 a,b) Size, (Lorica) 6$\mu$ high by 8$\mu$ broad. Cell almost fills the hyaline to golden brown, hemispherical lorica. The lorica is thick-walled (2 to 3$\mu$) and gelatinous with a flattened or somewhat concave base. Protoplasmic collar 4 to 5$\mu$ high. Flagellum 5 to 7$\mu$ long.

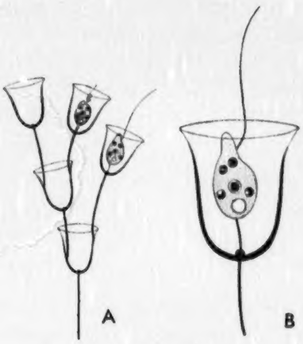
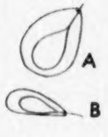


Fig. 107 *Poteriodendron petiolatum*
a. Colony
b. Detail of single cell



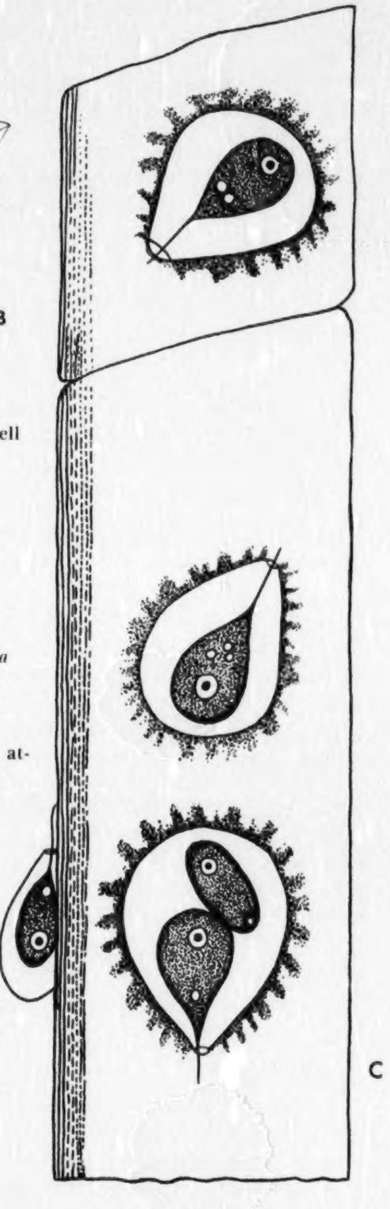


Fig. 108 *Platytheca micropora*
a. Outline
b. Outline–lateral view
c. Detail of several attached to a plant stalk

*Genus Poteriodendron*: Organisms with colorless to brown, almost conical (campanulate or bell-shaped), stemmed loricae which form arboroid colonies by cementing the stem of a new lorica to the inside surface of the previous lorica. The loricae usually show a basal thickening. Cells are usually spherical, elliptical or oval, and occupy only the bottom of the loricae where they are attached by a short contractile stem.

*P. petiolatum* Stein: (Fig. 107 a,b) Size, the literature records loricae 17 to 50μ high and cells 21 to 35μ long, but limited personal experience indicates that the cell normally lies entirely within the lorica. Therefore, 10 to 12μ or less may be the lower size limit of the cells, and these smaller sizes are the ones usually encountered in sewage treatment processes. Cells observed have been spherical, and it is possible that they may extend themselves to the dimensions recorded in the literature. A funnel-like mouth area extends forward, or laterally forward, alongside the flagellum. Flagellum almost twice body length. The colonies are apparently fragile and only single loricae, with stems, are usually seen.

*Genus Platytheca*: Cell contained in a flat lorica which is attached along its side. One or more contractile vacuoles. Division takes place within the lorica.

*P. micropora* Stein: (Fig. 108 a,b,c) Size, 12 to 18μ. Lorica, yellow-brown, pyriform in shape, with a narrow orifice. Cell almost fills lorica. Flagellum short, about ½ body length. Nucleus posterior. Vacuole in anterior end.

**Suborder Dimastigina,** suborder novum: Small organisms with two flagella, usually with very plastic bodies. Short, blunt, wide pseudopodia are frequently utilized in ingesting food. Food usually taken in through a specific body area. Flagella may be equal or unequal; both may be swimming flagella, or one may trail. In one case (*Rhynchomonas*), the anterior flagellum is replaced by an active, flexible, proboscislike, protoplasmic process.

FAMILY AMPHIMONADIDAE: The organisms have two equal flagella. Some dwell in loricae; others are naked. Naked types may be attached. Colonies are formed in some genera.

*Genus Amphimonas*: Naked cells with two equal flagella. Free swimming or attached to thin stems. One or two contractile vacuoles. Food taken in at base of flagella.

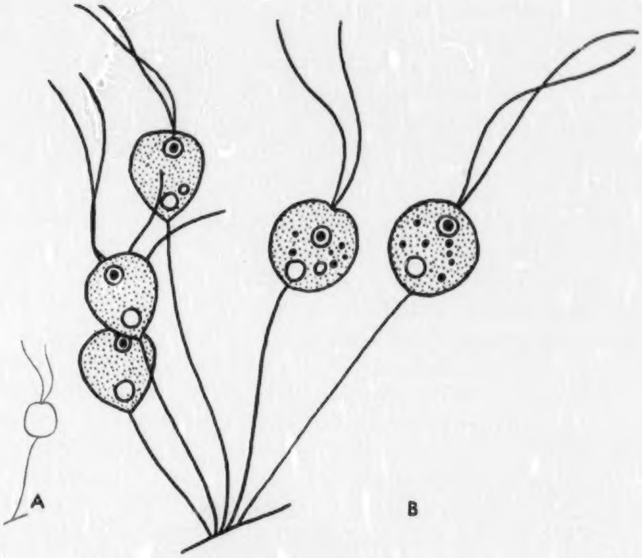


Fig. 109 *Amphimonas globosa*
a. Outline
b. Detail of a group of cells

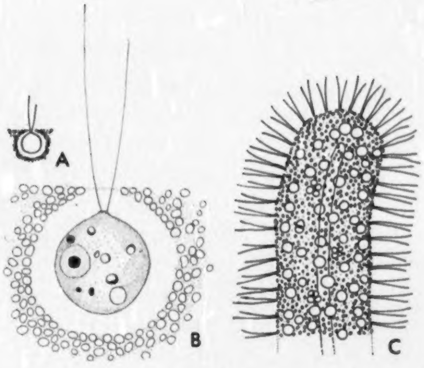


Fig. 110 *Spongomonas intestinum*
a. Outline of a single cell inbedded in matrix
b. Detail of a single cell
c. Colony

*A. globosa* S. Kent: (Fig. 109 a,b) Size approximately 12.5μ. Cells spherical on a long thin stem. Flagella 2 to 3 times body length. Two contractile vacuoles located posteriorly.

*A. fusiformis* Mez: (No illustration available) Size, 7 to 10μ by about ½ as broad. Cells free swimming, spindle-shaped. Flagella somewhat longer than the body. Vacuoles not recorded. Has been reported from sugar mill wastes.

*Genus Spongomonas*: Cells are embedded in a granular, gelatinous matrix which is sometimes branched. One contractile vacuole.

*S. intestinum* (Cienp.) S. Kent: (Fig. 110 a,b,c) Size cells, 8μ. Colonies wormlike and contorted, may be as large as 3 cm. long and 100 to 200μ broad. Interior of the colony sometimes hollow. Cells spherical. Flagella 2 to 3 times body length. Contractile vacuole posterior.

FAMILY BODINIDAE: Solitary, naked flagellates which are well represented in the sewage treatment biota. With unequal flagella or with one flagellum trailing (one genus with a flexible proboscis instead of an anterior flagellum). Pseudopodia are common, but are usually short, broad and blunt and often tend to be confined to a limited area of the body. Pseudopodia ordinarily important in taking food. Morphology is very variable in most species and seems to be affected by environment (chemical). The bodies do, however, have a basic shape.

*Genus Bodo*: Cells naked, free swimming, with one swimming and one trailing flagellum. Each flagellum has a basal granule and is attached by means of a rhizoplast to a parabasal body (kinetonuclear apparatus). Contractile vacuole, usually one, but 2 or 3 are sometimes present, seldom absent. Cysts are usually formed. The classification of *Bodo* is sorely in need of investigation. Generic characteristics are not well defined and this, associated with the wide variability within the genus, has led to serious difficulties. Morphology is a poor characteristic on which to base identification in this group, in spite of the fact that, in some species, morphology is relatively constant (see *B. glissans* and *B. putrinus*). Size varies with nutrition and perhaps with other environmental influences; as a result, size may not be significant (see *B. mutabilis*). The small size of the organisms, and the fact that original describers often failed to determine adequately, or describe, important differential

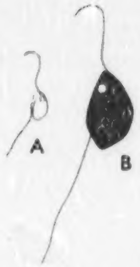


**Fig. 111** *Bodo alexeieffii*
a. Outline
b. Detail

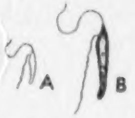


**Fig. 112** *Bodo angustus*
a. Outline
b. Detail

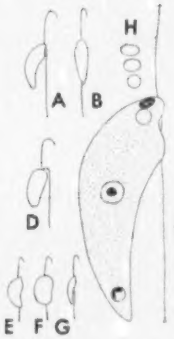


**Fig. 113** *Bodo caudatus*
a. Outline of a fairly typical cell in lateral (usual) view
b. Outline of dorsal view (this view is rarely seen)
c. Detail showing contractile vacuole and leucosin body
d,e,f,g. Outlines showing variations in morphology
h. Variations on cross section (the uppermost is the common one)

characteristics (such as kinetonuclei, type of motion, and cyst formation), leave some problems for which no answers can ever be obtained. There is little doubt that some organisms have been described under more than one specific name (see *B. mutabilis*). There is also little doubt that some species of *Bodo* have not yet been described. The previously mentioned small size of the organisms of the group and the difficulty in mechanically handling them are the chief contributing causes for this lack. Pascher (12) infers, and Calkins (2) definitely states, that this group has kinetonuclei, but a number of species are included which are not known to conform in this characteristic. Because of their number and activity in sewage treatment processes, it is desirable that a better understanding of the group should be obtained.

The members of the genus *Bodo* frequently lie on their sides and show distinct asymmetry as normally viewed. When they are thus found, the dorsal and ventral sides appear as the lateral sides of the organism rather than as the sides closest and farthermost, respectively, from the observer, as is frequently the case with other organisms. This should be kept in mind in examining these flagellates in order that expressions like, "the flagella originate slightly back of the anterior and ventral in position (or under the anterior)," may not leave the examiner with the impression that the flagella originate under the organism as he is viewing it; but on the contrary, come out of what, to the observer, is the side.

*B. alexeieffii* Lemmermann: (Fig. 111 a,b) Size, 6 to 12μ by 4 to 6μ. Cells broadly pyriform with ends distinctly reduced and pointed. Dorsal side strongly arched. Ventral side not furrowed. Swimming flagellum 1⅓, trailing flagellum 2, times body length. Kinetonucleus toward the dorsal side. Contractile vacuole opposite it and somewhat closer to the anterior end. Cysts not known.

*B. angustus* (Dujardin) Butschlii: (Fig. 112 a,b) Size, 8 to 12μ by 1.5 to 3μ Cells lancetlike, frequently spirally twisted. Flagella originate in a groove slightly back of the anterior end. Swimming flagellum ½ and trailing flagellum 1½ times body length. One contractile vacuole located anteriorly.

*B. caudatus* Dujardin: (Fig. 113 a,b,c,d,e,f,g,h) Size, 11 to 22μ by 5 to 10μ. Cells variable in shape but oval to almost inverted pyriform. The form with the posterior reduced in size is most typical. The body is usually somewhat curved. Pascher (12) states that this *Bodo* is strongly flattened; but, while it almost invariably lies on one side or the other, it may at times have a practically circular cross section. Considering the more curved side to be the dorsal side, the ventral side is characterized by a blunt, anterior ventral point; just back of which a slight depression may usually be found in the ventral margin and then a low, rounded outward swelling. The swollen area is the region in which ingestion of food takes place. The anterior flagellum has a hooking action and a "J"-like shape. This shape and action are so characteristic that forms with atypical morphology, which are so commonly found, may be identified because of the typical flagellar pattern. This anterior flagellum is almost body length. The trailing flagellum is somewhat longer (about 1½ times body length). Kineto-

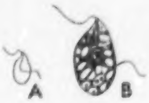


Fig. 114 *Bodo cruzi*
a. Outline
b. Detail

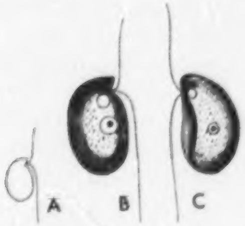


Fig. 115 *Bodo edax*
a. Outline—lateral (usual) view
b. Detail—"apparent" dorsal view
c. Detail—"apparent" ventral view

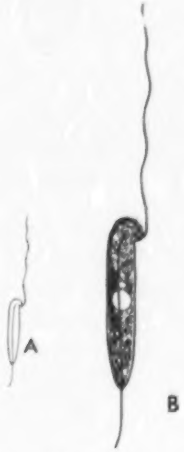


Fig. 116 *Bodo glissans*
a. Outline
b. Detail

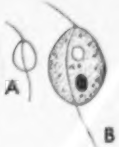


Fig. 117 *Bodo globosus*
a. Outline
b. Detail

nucleus present. A single contractile vacuole lies at the base of the flagella. Leucosin frequently present near the posterior region of the tail. Cytoplasm almost clear to very slightly granular. Spherical cysts without separate sheath known. The organism jerks along and may remain on one side for a long period of time. It practically never travels on the ventral side and very rarely swims freely.

*B. cruzi* (Hartmann et Chagas) Lemmermann: (Fig. 114 a,b) Size, 8 to 12$\mu$ by 5 to 6$\mu$. Cells pyriform, seldom broad spindleform and not metabolic. Swimming flagellum ½, trailing flagellum somewhat greater than, body length. Kinetonucleus present. Contractile vacuole absent. Cysts are formed.

*B. edax* Klebs: (Fig. 115 a,b,c) Size, 11 to 15μ by 5 to 7μ. Cells pyriform, anterior pointed like a bill. The more arched side may be considered the dorsal side. Ventral side deeply furrowed. Flagella originate on ventral side somewhat back of anterior end. Swimming flagellum 1⅓, trailing flagellum almost double, body length. Contractile vacuole anterior.

*B. glissans* Lackey: (Fig. 116 a,b) Size, 20μ or less. Cells somewhat flattened and elongated. Anterior rounded with a liplike fold. Posterior bluntly pointed. Sides parallel but organism very plastic, and bends readily. Swimming flagellum very fine and very active; almost impossible to observe on live specimens. It is about body length. Trailing flagellum thicker, somewhat longer, than the body and used as an axis on which the animal glides. Cytoplasm with light refractive granules giving the animal the appearance of a thick, short filament of the sulfur bacterium, *Beggiatoa*. Contractile vacuole median. Kinetonucleus and nucleus not seen. Movement steady, fairly rapid, straightforward on the substrate. May turn at a distinct angle and move off in a new direction. Nutrition saprophytic. Not found in strongly aerobic environments. Found in digestion tanks (Lackey), and sludge drawn from secondary tanks (Calaway).

*B. globosus* Stein: (Fig. 117 a,b) Size, 9 to 13μ by 8 to 12μ. Cells spherical to broadly pyriform. Swimming flagellum body length (Pascher), or somewhat shorter (Calaway). Trailing flagellum twice body length. One contractile vacuole slightly forward of the middle. The swimming flagellum strikes ahead and then swings back on the left (as seen in a microscope) covering an arc of about 90 degrees. The flagellum is more or less stiff and its beat is not too rapid for ready observation. Its action gives the organism a side-to-side rocking motion as it moves rather steadily along. The organism glides on the substrate with its ventral side and origin of flagella down. Our experience indicates this is the second most common *Bodo* of sewage treatment (*B. caudatus* is first).

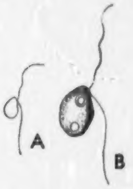


Fig. 118 *Bodo lens*
a. Outline
b. Detail

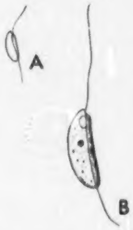


Fig. 119 *Bodo mutabilis*
a. Outline
b. Detail

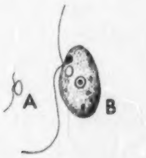


Fig. 120 *Bodo minimus*
a. Outline
b. Detail

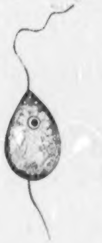


Fig. 121 *Bodo ovatus*

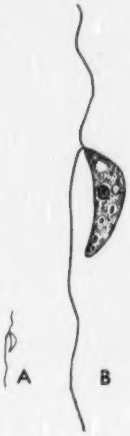


Fig. 122 *Bodo putrinus*
a. Outline
b. Detail

*B. lens* (Muller) Klebs: (Fig. 118 a,b) Size, 6 to 7$\mu$ long. Cells spherical to pyriform. Flagella originating laterally and underneath the anterior border. They are almost equal in length being 2 to 2½ times the cell length. One posterior contractile vacuole. Motion rotating or, when fastened, swaying like a pendulum.

*B. mutabilis* Klebs: (Fig. 119 a,b) Size, 8 to 14$\mu$ by 3 to 5$\mu$. Cells almost cylindrical but somewhat flattened with rounded ends. Flagella originate in a groove slightly back of, and underneath, the anterior margin. Flagella of almost equal length, 1½ times body length. Contractile vacuole in anterior end. Movement by jerky swimming.

The description given here conforms to the description of an environmentally produced variant of *B. caudatus*, observed by Calaway. The size of the cells of this "species" overlaps the size of *caudatus.* The rounded posterior shown by Pascher (12) is similar to the atypical but very common form shown in the illustration for *B. caudatus.* The jerky motion, the anterior contractile vacuole and flattening all agree with *caudatus.* The origin of the flagella and the slight differences in length present no problem. Since the kinetonucleus is "not proven" (Pascher), there is no cause for disagreement on this structure. It is also possible that *B. alexeieffii* Lemermann, and remotely possible that *B. minimus* Klebs, are small *B. caudatus.* In all these, even as in *B. caudatus,* the descriptions leave something to be desired because of the omission of certain characteristics or because of the possibility of interpreting some characteristics to conform to descriptions of other organisms.

*B. minimus* Klebs: (Fig. 120 a,b) Size, 4 to 5μ by 2 to 2.5μ. Cells thick, bean-shaped. Flagella originate in a groove slightly back of, and underneath, the forward end. Swimming flagellum body length, trailing flagellum twice as long. Kinetonucleus present. One anterior contractile vacuole underneath the base of the flagella. Motion slowly creeping.

*B. ovatus* (Dujardin) Stein: (Fig. 121) Size, 27 to 35μ. Cells pyriform with anterior end pointed, less frequently inverted pyriform. Swimming flagellum greater than body length and originating on the anterior tip. Trailing flagellum twice as long as body and springing from ventral side slightly back of the anterior tip. Nucleus in the anterior half of the cell. One or possibly three contractile vacuoles in the anterior end.

*B. putrinus* (Stokes) Lemmermann: (Fig. 122 a,b) Size, 5 to 8μ. Cells inverted pyriform, somewhat bent. They have the appearance of a miniature *B. caudatus* but are apparently never laterally flattened. The body form is quite constant. Swimming flagellum somewhat more than body length. Trailing flagellum 3 times body length. Motion rotating, zig-zag in manner; if attached, then with a circling motion. The swimming motion is quite characteristic since the anterior lateral prominence may be seen first on one side and then on the other, giving an almost flip-flop appearing motion. These organisms swim freely but rarely at any

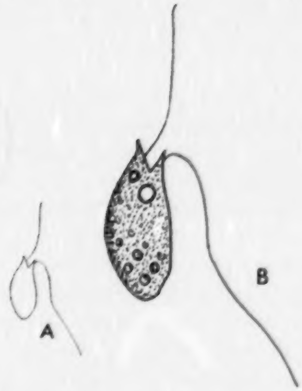


Fig. 123 *Bodo saltans*
a. Outline
b. Detail

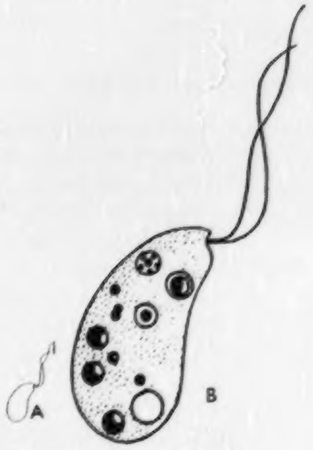


Fig. 124 *Dinomonas vorax*
a. Outline
b. Detail

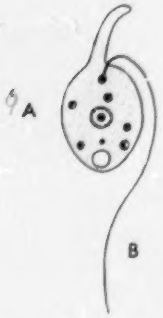


Fig. 125 *Rhynchomonas nasuta*
a. Outline
b. Detail

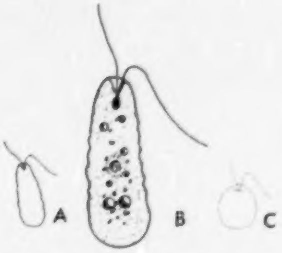


Fig. 126 *Phyllomitus amylophagus*
a. Outline
b. Detail
c. Outline of a contracted cell

great distance above the substrate. Personally, we have never encountered this organism in raw sewage but it frequently is numerous in environments that produce increases in *B. caudatus.*

*B. saltans* Ehrenberg: (Fig. 123 a,b) Size, 17 to 21$\mu$. Cells pyriform. Flagella arise from a groove or depression just back of the anterior end. This groove elongates into a weakly screw-shaped ventral furrow. Swimming flagellum body length, trailing flagellum 2 to 3 times as long. Kinetonucleus present. Contractile vacuole anterior. Cysts not known.

*Genus Dinomonas*: Cells free swimming with two flagella almost equal in length and active in a generally anterior direction. One contractile vacuole in the posterior end. Kinetonucleus absent. Cysts known. Nutrition holozoic, feeds on *Bodo*.

*D. vorax* S. Kent: (Fig. 124 a,b) Size, 15 to 20μ. Cells pyriform, anterior pointed and slightly bent. Surface smooth. Flagella more than body length.

*Genus Rhynchomonas*: Cells naked with an antero-lateral depression (mouth area) from which the trailing flagellum originates. In place of the swimming flagellum, there is present an active proboscislike protoplasmic extension. One contractile vacuole is present in the anterior end.

*R. nasuta* (Stokes) Klebs: (Fig. 125 a,b) Size, 5 to 6μ by 2 to 3μ. Cells elliptical to pyriform, somewhat flattened. Anterior protoplasmic extension about body length. Trailing flagellum twice as long. The organism moves on the substrate in an active manner, first on one side and then on the other. This flipping from one side to the other, after one to several strokes of the proboscis, is quite characteristic and aids in rapid recognition of the organism after a few observations.

*Genus Phyllomitus*: Cells naked, very metabolic with a pharynxlike depression (mouth area). One swimming and one trailing flagellum, each with a basal granule. One contractile vacuole in the anterior. Cysts not known.

*Ph. amylophagus* Klebs: (Fig. 126 a,b,c) Size, 19 to 25μ by 7 to 13μ. Cells almost cylindrical and somewhat flattened. Anterior truncated. Trailing flagellum slightly longer than the swimming flagellum. Contractile vacuole anterior. Known to ingest starch granules.

*Genus Bodopsis*: Cells naked and free swimming. One swimming flagellum and one trailing flagellum, each springing from wide, shallow, pharynxlike mouth opening. Nucleus with a karyosome. Nutrition saprophytic and holozoic. Pseudopodia spring from mouth area and extend only laterally. They are broad with branched ends. Cysts not known.

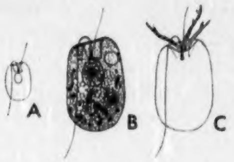


Fig. 127 *Bodopsis godboldi*
- a. Outline
- b. Detail
- c. Showing pseudopods emerging from mouth area

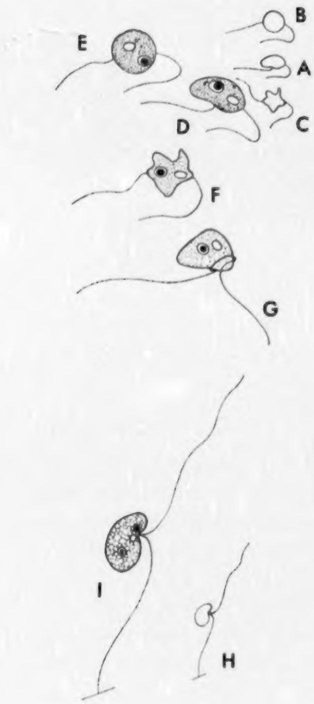


Fig. 128 *Pleuromonas jaculans*
- a. Outline of attached form
- b. Outline of free swimming individual
- c. Outline of amoeboid form
- d, e, f. Detail of forms a, b, and c
- g. Detail of form frequently seen by one of the writers
- h. Outline of the form usually seen
- i. Detail of form usually seen

*B. godboldi* Lackey: (Fig. 127 a,b,c) Size, average 12μ by 6μ by 3 to 4μ thick. Cells generally ovate in outline and dorsoventrally bowed, but considerable deviation from this shape may be seen. Individuals constant in shape. Pellicle thick and unmarked. Has a typical bowl-shaped, anterior depressed mouth area from which two flagella, and at times pseudopodia, extend. Anterior flagellum ¼ body length and vibratile. Trailing flagellum 1½ times body length. Contractile vacuole adjacent to, and emptying into, the mouth depression. Probably creeps on substrate with aid of anterior flagellum. Often motionless for a long time then moves with a slightly jerky motion. Not known to swim freely. Cytoplasm is clear and uniform. Food vacuoles are at times present and undergo slow cyclosis. Bright spheres of unknown nature are usually present in the posterior end of the cell. Nucleus lies just under the bottom of the mouth depression and possesses a large endosome. Basal granules of the flagella rest on the distinct nuclear membrane, and the nucleus is slightly pointed in this direction. Two to six pseudopodia emerge from the mouth area at times. They are fine but possess swellings. They seldom branch and do not anastomose. The swellings are active in capturing bacteria. The pseudopodia rarely exceed twice the body length and do not possess axial filaments.

*Genus Pleuromonas*: Cells naked, weakly amoeboid and usually attached to substrate by the trailing flagellum. Protoplast with vigorous streaming. One contractile vacuole anterior. Nutrition holozoic, food taken into a preformed vacuole in the anterior dorsal area. Cysts known.

*Pl. jaculans* Perty: (Fig. 128 a,b,c,d,e,f,g,h,i) Size, 6 to 10μ by 5μ. Body bean-shaped to spherical but very irregular. All cells will have a fairly uniform shape but may differ widely from this shape at another time. Sewage seems to be a very satisfactory environment for these organisms and, when conditions are ideal, they may exceed the above size by as much as 2 times. Flagella 2 to 3 times body length. Pascher (12) states that the anterior swimming flagellum originates from the anterior end and the trailing flagellum from a lateral (actually ventral) swelling. Our observations indicate that both flagella originate from this same lateral area, and that the anterior flagellum is loosely coiled over the body surface

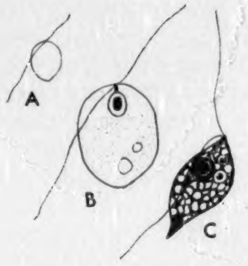


Fig. 129 *Cercobodo crassicauda*
a. Outline
b. Detail
c. Detail of a slightly different form

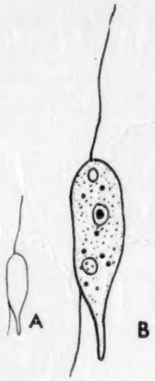


Fig. 130 *Cercobodo longicauda*
a. Outline
b. Detail

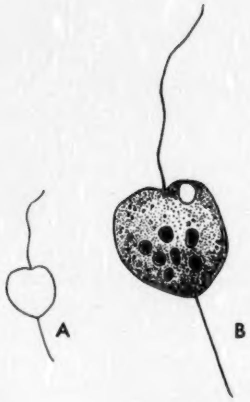


Fig. 131 *Cercobodo ovatus*
a. Outline
b. Detail

but that it passes from the origin to the anterior face where it can frequently be seen to move (*see illustration*). One contractile vacuole dorsal or posterior but may be variable. Motion pitching and tossing while anchored by the trailing flagellum. This organism has the nickname "jumping monad," which is quite descriptive. When free swimming (they ordinarily swim to seek higher oxygen levels), they are more difficult to identify than the attached forms.

*Genus Cercobodo*: Cells naked, amoeboid, free swimming or creeping amoebalike. They have one swimming and one trailing flagellum. Each flagellum has a basal granule, and each basal granule is connected to the nucleus by a rhizoplast (very difficult to determine). Contractile vacuoles, one to many. Nutrition saprophytic or holozoic by means of pseudopodia. Cysts with firm membranes. Pascher (12) states in a section apart from the description of this genus that it may be distinguished from *Bodo* by the strong metaboly of its posterior end. Calkins (2) states that the trailing flagellum adheres to the entire length of the body. Irregular pseudopodia arise from the posterior portion and from the ventral side. While the flagellum does adhere, it may also probably be free at times. This genus now contains the organisms some of the older writers designated as *Cercomonas*.

*C. crassicauda* (Alexeieff) Lemmermann: (Fig. 129 a,b,c) Size, 12 to 16$\mu$ by 7 to 10$\mu$. Cells spindleform with posterior drawn out into a short tail. Flagella approximately 1¼ body length; swimming flagellum slightly shorter than trailing flagellum. Contractile vacuole. Nucleus with large karyosome. Cysts spherical 9 to 11$\mu$.

*C. longicauda* (Stein) Lemmermann: (Fig. 130 a,b) Size, 18 to 36$\mu$ by 9 to 14$\mu$. Cells inverted pyriform to spindle-shaped. Posterior end drawn out tail-like. Flagella equally long, about body length. Amoebalike with posterior, thick or thin, single or branched, pseudopodialike extensions. One or two contractile vacuoles in the posterior end. Nutrition holozoic or saprophytic.

*C. ovatus* (Klebs) Lemmermann: (Fig. 131 a,b) Size, 15 to 19$\mu$ by 18 to 21$\mu$ broad. Cells frequently broader than long, broadly pyriform but usually rounded. Posterior somewhat reduced. Flagella equally long and double body length. One

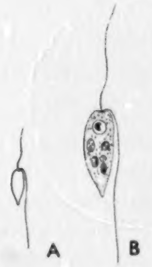


**Fig. 132** *Cercobodo radiatus*
a. Outline
b. Detail

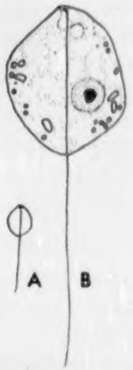


**Fig. 133** *Clautriavia parva*
a. Outline
b. Detail

anterior contractile vacuole. When amoeboid, it has broad, blunt pseudopodia. Nutrition saprophytic or holozoic.

*C. radiatus* (Klebs) Lemmermann: (Fig. 132 a,b) Size, 10 to 14$\mu$ by 5 to 9$\mu$. Cells elongated pyriform, less frequently inverted pyriform. Swimming flagellum body length, trailing flagellum double body length. One posterior contractile vacuole. Pseudopodial processes long, finely pointed, radially out-reaching. Nutrition holozoic and saprophytic.

Two other organisms reported from sewage treatment apparently belong in this order or in some previous order. These are the *Clautriavia* and *Helkesimastix* flagellates on which only the trailing flagellum has been distinguished. Their relation to other organisms is obscure. Hollande (5) suggests that *Clautriavia* is a euglenoid. It may be that *Helkesimastix* and *Clautriavia* are synonyms.

*Genus Clautriavia*: Colorless cells with distinct periplast. The cell possesses, on its anterior ventral side, a trough-shaped mouth area, from the bottom of which the flagellum emerges. Small contractile vacuole in the mouth area empties into a reservoirlike structure. Nucleus posterior. Stored food product paramylum. Nutrition holozoic. Motion, jerkily forward with the anterior end gliding along the substrate and the posterior end oblique or perpendicular.

> *C. parva* Schouteden: (Fig. 133 a,b) Size, approximately 10$\mu$. Cells asymmetrically pyriform with anterior noticeably smaller than the posterior which is broadly rounded. Mouth area not drawn out into a furrow. Flagella about 4 times body length. Nucleus in the posterior left quarter.

*Genus Helkesimastix*: Small plastic organisms, usually elongated pyriform to cylindrical in shape, but sometimes oval or pyriform. The anterior is rounded and the posterior tapers and is occasionally pointed or drawn out like a tail. The greatest width is usually well forward. They possess only a trailing flagellum which adheres to the body along at least ⅓ to ½ the body length. The flagellum is not attached, however. This flagellum originates anteriorly (except in freshly excysted forms in which it is nearer the middle). It passes dorsally along the body and Woodcock and Lapage (20) state that the flagellum is never ventral under ordinary conditions. These organisms move rather rapidly, considering the lack of an active flagellum, and the means of locomotion is not understood. A characteristic movement of the anterior (jerking from side to side) may sometimes be seen but is never of very great amplitude. They rarely swim freely, but when they do so, it is by an undulating motion of the body. The nucleus lies just back of the anterior margin and is typically vesicular. It does not have a kinetonucleus. The cytoplasm is finely granular and dots or punctae may frequently be present along the flagellum (species difference?). They are, undoubtedly, largely saprozoic.

Only one species has been described from sewage treatment processes but the original describers mentioned another species from goat and sheep dung from which the species below was first described. Differences in organisms of this genus may indicate that three or four species can be found in sewage treatment biota.

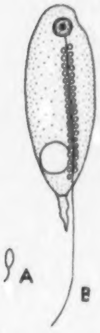


Fig. 134 *Helkesimastix faecicola*
a. Outline
b. Detail of an individual with many granules along the flagellum

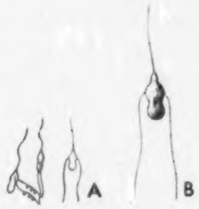


Fig. 135 *Dallingeria drysdali*
a. Outline of attached cell
b. Detail of free-swimming cell

*H. faecicola* Woodcock and Lapage: (Fig. 134 a,b) Size, 6 to 7$\mu$. Usually elongated oval or pyriform to nearly cylindrical with a rounded anterior end and a reduced and sometimes pointed, almost amoeboid, posterior. The organism has a very delicate pellicle. The single flagellum is trailing and lies along the middle of the dorsal side. The original describers mentioned the frequent presence of 3 or 4 conspicuous granules in a row along the flagellum. The plasma undergoes amoeboid changes as the animal reverses its direction of movement by doubling back on itself in a very characteristic manner. The posterior contractile vacuole is proportionately very small. Division is preceded by withdrawal or absorption of the flagellum followed by the separation of two approximately equal portions of the protoplast; first by formation of a dumb-bell shape, and then the gradual separation of the ends of the dumb-bell as new flagella begin to appear. The division appears to take place in the transverse plane but the flagella reappear at the outer extremes. Conjugation and encystment are known.

**Order Polymastigida:** Organisms with 3 to 8 or more flagella. (No organism reported from sewage is known to have more than 8 flagella.) This is a mixed group and there are no clear-cut relationships among the various families and often among the genera of the families. It may, therefore, be expected that some noticeable changes will occur in the classification of organisms in this order. The group is divided here on the basis of the number of flagella; a convenient, if not entirely logical, basis.

FAMILY TRIMASTIGIDAE: Organisms with one anterior, and two trailing, flagella. Sometimes attached by the trailing flagella. Pellicle layer delicate. One contractile vacuole.

*Genus Dallingeria*: Cells free swimming or attached by means of the trailing flagella. Anterior of cell with a necklike constriction. Swimming flagellum originates from the anterior tip or under it; trailing flagella posterior to the necklike constriction and from opposite sides of the body. Contractile vacuole. Cysts produced. Sexual reproduction has been reported.

*D. drysdali* S. Kent: (Fig. 135 a,b) Size, 6.5$\mu$. Cells elongated pyriform, anterior pointed. Swimming flagellum 1¼ to 2 times body length; trailing flagella 2 times body length.

FAMILY TETRAMITIDAE: Flagellates with 4 equal or unequal flagella, one or two of which may be trailing. Cells are free swimming or attached by means of the flagella. Pellicular layer is delicate. No parabasal body or axostyle has been reported, but a rhizostyle is present in *Tetramitus* and one other genus. One contractile vacuole.

*Genus Tetramitus*: Cells free swimming, slightly metabolic. Mouth area a groove or furrow. Four unequal flagella, some swimming and some trailing. One contractile vacuole and usually several food vacuoles. Cysts not known. An amoeboid phase has been reported for members of this genus and, on the strength of this evidence, Hollande (5) transferred them to the *Sarcodina*. However, this present classification (which follows Pascher) is retained for convenience and because the flagellate stage appears to be dominant in sewage.

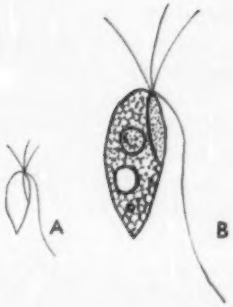


Fig. 136 *Tetramitus decissus*
a. Outline
b. Detail

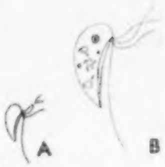


Fig. 137 *Tetramitus pyriformis*
a. Outline
b. Detail

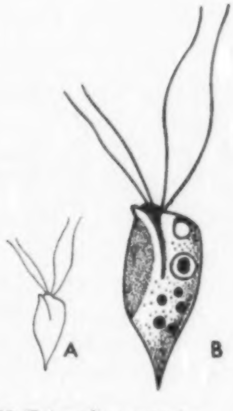


Fig. 138 *Tetramitus rostratus*
a. Outline
b. Detail

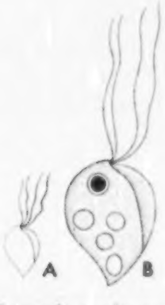


Fig. 139 *Tetramitus sulcatus*
a. Outline
b. Detail

*T. decissus* Perty: (Fig. 136 a,b) Size, 13 to 28μ by 7 to 15μ. Cells spindleform with anterior lateral border, sharply truncated to produce a broad, oval, depressed mouth area. Posterior edge of mouth area may reach slightly beyond the mid-point. Flagella originate on the anterior end and consist of 3 short swimming ones and a fourth longer trailing flagellum. Contractile vacuole posterior. Common in anaerobic conditions.

*T. pyriformis*: (Fig. 137 a,b) Size, 11 to 13μ by 10 to 12μ. Cells broadly inverted pyriform with a curved appearance. Posterior end bluntly pointed. Flagella originate laterally on the anterior margin, and the mouth area extends from the flagella insertions to the posterior end and is set off with well-marked edges. Flagella unequal in length with the longest trailing and the others directed anteriorly (Pascher). We have observed this species when only the trailing and 1 anterior flagellum could be easily distinguished. The other 2 flagella were found to be held close against the mouth area. Contractile vacuole posterior. This is the most common *Tetramitus* of sewage treatment according to present experience.

*T. rostratus* Perty: (Fig. 138 a,b) Size, 18 to 30μ by 8 to 11μ. Cells inverted pyriform with posterior tapering to a point. Shape very variable, often flattened and/or angular and/or with a membranelike extension on one side. Anterior almost transversely truncated and with a lateral fissure which forms a bill-like prominence. Mouth area oval, reaching into posterior half. Two longer and 2 shorter flagella are all active in swimming and arising apically. Contractile vacuole anterior and usually prominent.

*T. sulcatus* Klebs: (Fig. 139 a,b) Size, 17μ by 15μ. Cells broadly inverted pyriform with posterior bluntly pointed. Mouth area, broad, oval, with a slight oblique twist around the body and reaching almost to the posterior end. Two long and 2 short swimming flagella. Contractile vacuole in posterior end.

*Genus Collodictyon*: Since the taxonomic position of this organism is highly unsatisfactory, its placement here is only tentative. Cells strongly metabolic with several longitudinal fur-

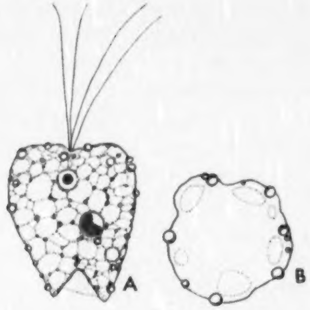


Fig. 140 *Collodictyon triciliatum*
a. Longitudinal view
b. Transverse view

rows; posterior end extends into one or more tips. Without distinct mouth area. Four swimming flagella. One contractile vacuole anterior, but larger individuals lack contractile vacuoles and food vacuoles. Nutrition holozoic. Food taken in at any point on body surface. Motion rotating. Cysts not known.

*C. triciliatum* Carter: (Fig. 140 a,b) Size, 27 to 60$\mu$. Cells inverted pyriform, heart-shaped or spherical. Posterior sometimes with pseudopodia. Anterior broadly rounded or almost squared off. Flagella originate in a small anterior beak and are body length. A number of clear, small vacuoles

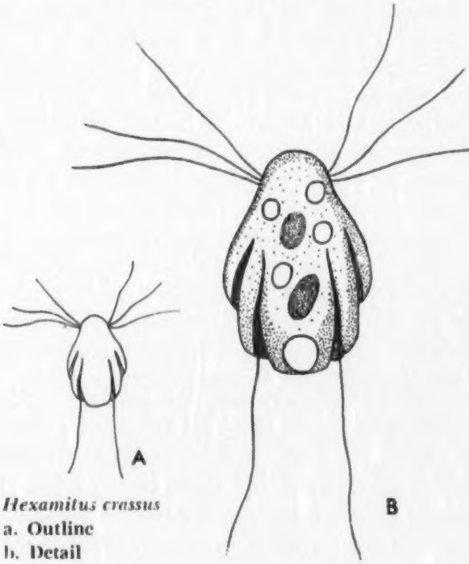


Fig. 141 *Hexamitus crassus*
a. Outline
b. Detail

underlie the delicate pellicle causing distinct hemispherical bumps on the surface of the organism.

FAMILY HEXAMITIDAE: Members of this family are apparently made up of two fused cells since two nuclei are present, the flagella originate similarly on opposite sides of the body and are always present in even numbers, and other structures are commonly present in duplicate. These facts evidently were the reason for Pascher's (12) classifying them in a separate order (*Distomatinae*—two-mouthed). This seems to be a logical classification but has not been adhered to by other authors. Calkins (2) evidently recognized certain difficulties and divided the order *Polymastigida* into Group *Monozoa* and Group *Diplozoa* (two animals) as a kind of noncommittal classification. Calkin's *Diplozoa* and Pascher's *Distomatinae* included practically the same genera. Kudo (8) classified them under Suborder *Diplomonadina* and considers the other suborder to be *Monomonadina*. Bilateral symmetry is characteristic of the family. Parabasal bodies and axostyles are present in at least some genera. One or two contractile vacuoles. Four to several flagella (always of an even number). Stored food, fat or oil, possibly at times a carbohydratelike substance also.

*Genus Hexamitus*: Cells with delicate pellicle layer, free swimming, and each side with a mouth slit (not readily discovered). Six swimming flagella originate in the neighborhood of the anterior end, but as two opposite groups of three flagella each. The two trailing flagella originate in the mouth slits or in separate furrows, and these two trailing flagella form the characteristic by which this genus is most readily recognized. They occasionally twist loosely together. One or two contractile vacuoles usually form laterally and migrate posteriorly to discharge. Nuclei, two half-moon-shaped bodies with large endosomes; located anteriorly. Protoplasm often contains highly refractive spheres. Nutrition holozoic and saprophytic. Motion steadily rotating, or a noticeable jerking rotation of one, or a fraction, turns.

*H. crassus* Klebs: (Fig. 141 a,b) Size, 24 to 35$\mu$ by 14 to 18$\mu$. Cells pyriform, anterior reduced and broadly rounded. Mouth slit not reaching to posterior end. Trailing flagella extend beyond posterior end by up to ½ the cell length. Contractile vacuole discharges through the lateral border.

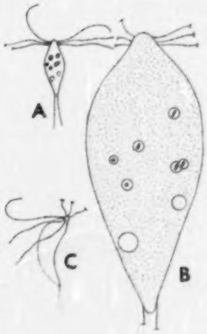


Fig. 142 *Hexamitus fissus*
a. Outline of a small cell
b. Detail of the small cell
c. Lateral outline of a medium sized cell showing the flexing that frequently occurs

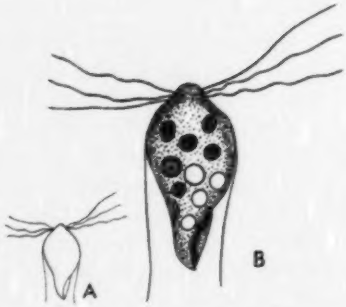


Fig. 143 *Hexamitus fusiformis*
a. Outline
b. Detail

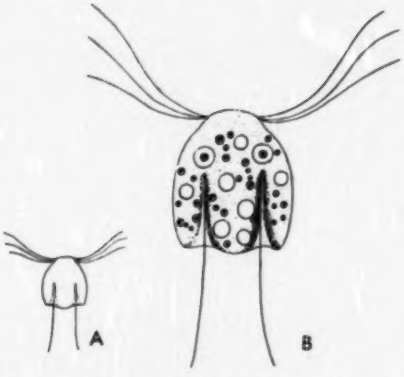


Fig. 144 *Hexamitus inflata*
a. Outline
b. Detail

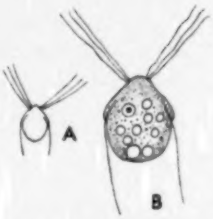


Fig. 145 *Hexamitus pusillus*
a. Outline
b. Detail

*H. fissus* Klebs: (Fig. 142 a,b,c) Size, 20 to 26μ by 9 to 13μ. Cells inverted pyriform, posterior pointed. Mouth slit broad and extending to base of posterior point. Trailing flagella extend about ½ cell length beyond the organism. Contractile vacuole discharges through the lateral border. Poly- to mesosaprobic.

*H. fusiformis* Klebs: (Fig. 143 a,b) Size, 22 to 27μ by 10 to 12μ. Cells cylindrical to spindleform; posterior sometimes rounded. Mouth slits approach the posterior end which is somewhat helically twisted. Trailing flagella reach only slightly beyond posterior end. Contractile vacuole discharges through the posterior end.

*H. inflata* Dujardin: (Fig. 144 a,b) Size, 13 to 25μ by 9 to 15μ. Cells broadly oval, posterior transversely truncated and somewhat rounded. Mouth slits reach to the vicinity of the posterior border. Flagella extend one to two cell lengths beyond the posterior end. The contractile vacuole discharges in the posterior region.

*H. pusillus* Klebs: (Fig. 145 a,b) Size, 10 to 13μ by 8 to 10μ. Cells pyriform, anterior strongly reduced. Mouth slits reach almost to the middle of the body. Trailing flagella extend one full cell length beyond the posterior. Trailing flagella widely separated, originating at about the widest part of the cell. Contractile vacuoles discharge through the posterior margin of the cell.

*Genus Trepomonas*: Cells with delicate pellicle layer. Free swimming. In cross section S-shaped and with a winged posterior. Each side has a pocket-shaped mouth area in which four flagella originate (pocket and some of the flagella almost impossible to discover according to our experience). There are two elongated nuclei located anteriorly, each with a distinct karyosome near its anterior end and reduced in size towards the posterior end, ending near the flagella insertions where a rhizoidlike structure springs from the basal granule which is applied to the nucleus (these structures cannot be recognized without staining). A single contractile vacuole migrates from the middle of the body toward the posterior to discharge. Forward motion steadily rotating, a jerking rotation of one turn, or a fraction of a turn; or springing.

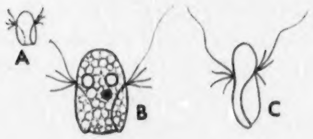


Fig. 146 *Trepomonas agilis*
a. Outline
b. Detail
c. Lateral view

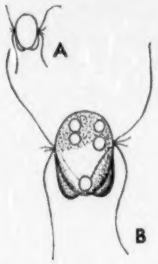


Fig. 147 *Trepomonas rotans*
a. Outline
b. Detail

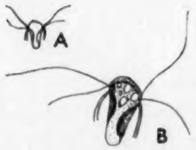


Fig. 148 *Trepomonas steinii*
a. Outline
b. Detail

*T. agilis* Dujardin: (Fig. 146 a,b,c) Size, 7 to 30μ Cells more or less pyriform but often strongly angular. The main portion of the body is reduced posteriorly. This portion forms an axis to which the winglike posterio-lateral lobes are attached. The anterior limit of these wings is in the area of the flagella insertions. These wings curve and give the organism the "S" appearance mentioned in the generic description; or, in certain views, the animal appears to have a comma (,) shape. One pair of long and 3 pairs of short flagella (hundreds of these organisms may be observed without seeing the short flagella). Motion rotating, frequently only ¼ turn with each stroke of the flagella, at other times swimming rapidly with rotation. This is perhaps the typical protozoan of raw sewage. It is found in Imhoff tanks, in digesters and in many other places if aerobic conditions do not prevail for long time intervals. It is anaerobic but will thrive under aerobic conditions if biological activity is intense.

*T. rotans* Klebs: (Fig. 147 a,b,) Size, 10 to 13μ by 7 to 8μ. Cells broadly oval, in the posterior half strongly flattened, but with the main posterior portion of the body thick and rounded rather than as a reduced, almost cylindrical, axis as in *agilis.* Mouth pockets flat, 2 pairs of long and 2 pairs of short flagella with the former somewhat more than body length. Motion rotating.

*T. steinii* Klebs: (Fig. 148 a,b) Size, 7 to 11μ by 3.5 to 6μ. Cells inverted pyriform and helically twisted. Two pairs of long and 2 pairs of short flagella, with the former more than double body length. Motion a jerking or almost springing rotation.

*Genus Trigonomonas*: Cells with delicate pellicle layer. Free swimming. Each side with a trough-shaped, somewhat spiral, mouth area (this is difficult to distinguish). Each body side with 3 swimming flagella originating underneath the anterior end. Only 1 flagellum on each side is readily observed. The others lie loosely along the body surface, but only one other on each side has been definitely seen. One contractile vacuole. Nucleus thick and located under the anterior end, biscuit-shaped (whether two nuclei are present is unknown). Cysts not known. Nutrition holozoic.

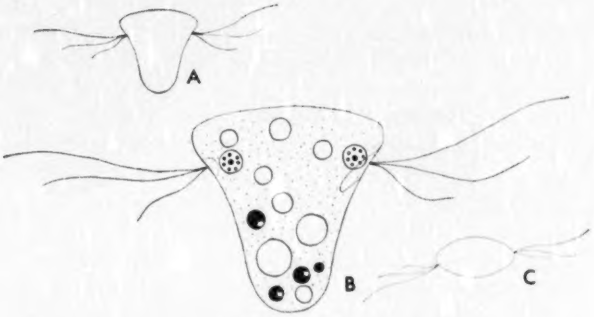


Fig. 149 *Trigonomonas compressa*
a. Outline
b. Detail
c. Anterior view

*T. compressa* Klebs: (Fig. 149 a,b,c) Size, 24 to 33$\mu$. Cells almost inverted pyriform with rounded ends, compressed. Flagella unequally long. Motion rotating or shaking side to side.

Kudo (8) pictured this species as being more triangular in shape with the narrowest side of the triangle anterior. This is agreed to, but the longer sides are somewhat concave and the angles always rounded. It would also be suggested that sizes in the neighborhood of 15 to 20$\mu$ are more commonly observed. The end view of the cell shows a rather strongly compressed elliptical outline.

*Genus Urophagus*: Pascher (12) suggests that this name is synonymous with *Hexamitus*. Hall (4) repeats this suggestion. It would, therefore, appear that no definite evidence for this as a separate genus has been presented in the last 40 years. Examination of the descriptions and drawings indicates that both are similar. The posterior "klappigen" might be interpreted as the posterior ends of the lips of some of the *Hexamitus* species. It seems possible that the name *Urophagus* might be suppressed and *Urophagus rostratus* be considered a variant of *Hexamitus fissus*.

# APPENDIX A

## SOLUTIONS USED IN MICROSCOPY

### 1. Noland's Stain

| | |
|---|---|
| Gentian violet | 20 mg |
| (Moisten with 1 ml of water) | |
| Phenol (saturated aqueous solution) | 80 ml |
| Formalin (38-40%, i.e., concentrated) | 20 ml |
| Glycerine | 4 ml |

### 2. Methyl Green-Acetic Acid

| | |
|---|---|
| Methyl Green | 0.5 g |
| Water | 100 ml |

Extract repeatedly with 10 ml quantities of chloroform discarding the purple chloroform layer each time until the chlorform is only slightly colored. (Methyl violet, an impurity in the methyl green which interferes with proper staining, is the material extracted.) Acidify the final watery layer with:

| | |
|---|---|
| Glacial acetic acid | 1 ml |

This method courtesy of Dr. L. C. Gilman of the University of Miami.

### 3. Neutral Red – a vital stain

This does not keep well as a dilute solution. The following stock solution keeps better.

| | |
|---|---|
| Neutral red | 1 g |
| 95% Ethyl alcohol | 100 ml |

The working solution can be prepared by diluting one drop of the stock solution with 49 drops of clear water from the sample. (Water clarified by settling, filtering or centrifuging.)

### 4. Methyl Cellulose

| | |
|---|---|
| Methyl cellulose | 10 g |
| Water | 90 ml |

Heat about one-half the water to boiling; add the solid and when completely dissolved, add the balance of the water.

5. Lugol's iodine solution for starch test (modified)

| | |
|---|---|
| Iodine (crystals) | 6 g |
| Potassium iodide | 4 g |
| Water | 100 ml |

This should be kept in a dark bottle, and some iodine crystals should be kept in contact with the solution at all times. The working solution should be diluted (as above—under neutral red) to 1/5 or 1/10 the strength of stock solution; i.e., 1 drop plus 4 drops of water or 1 drop plus 9 drops of water.

## APPENDIX B

## LENGTH, PIGMENTATION, AND COLOR FORMATION OF FLAGELLATES

The size used for grouping is the usual size of individuals (even when colonies are formed) except if the word "lorica" appears in parentheses after the name of the organism, then the size used is the length of the lorica. Under the column "Size Variation" a plus (+) sign indicates that the organism is frequently of the size of the next larger group, similarly a negative (−) sign indicates frequent occurrence of sizes falling in the next smaller group. Under "Pigmentation," G indicates green, Y - yellow, B - blue and Br - brown. Organisms which are unmarked in this column are normally colorless. Under the column headed "Colony" a plus (+) will indicate that the organism is almost always found as a colony, ± indicates that both colonies and single cells may be encountered.

| | Size Variation | Pigmentation | Colony |
|---|---|---|---|
| | Less than $20\mu$ | | |
| Chromulina ovalis | | Y-G | |
| Kephyrion ovum | | Br | |
| Oicomonas mutabilis | + | | |
| Oicomonas ocellata | | | + |
| Oicomonas socialis | | | ± |
| Oicomonas steinii | + | | |
| Oicomonas termo | | | |
| Monas amoebina | | | |
| Monas arhabdomonas | + | | |

| | Size Variation | Pigmentation | Colony |
|---|---|---|---|
| Monas minima | | | |
| Monas obliqua | | | |
| Monas vulgaris | + | | |
| Physomonas vestita | | | |
| Stylobryon abbottii (lorica) | + | | ± |
| Anthophysa vegetans | | | + |
| Chroomonas setoniensis | | B | |
| Chroomonas cyaneus | | B | |
| Polytoma citri | | | |
| Spermatozopsis exultans | | | |
| Lobomonas rostratus | | G | |
| Spondylomorum quarternarium | + | G | + |
| Gonium pectorale | | G | + |
| Gonium sociale | + | G | + |
| Pleodorina californica | | G | + |
| Phacotus lenticularis | + | G | |
| Trachelomonas voivocina (lorica) | + | G-Br | |
| Scytomonas pusilla | | | |
| Notosolenus apocamptus | | | |
| Notosolenus orbicularis | + | | |
| Anisonema ovale | | | |
| Anisonema emarginata | | | |
| Mastigamoeba reptans | | | |
| Mastigamoeba sp. (Lackey) III | | | |
| Codonosiga botrytis | + | | + |
| Monosiga ovatus | | | |
| Salpingoeca marssonii (lorica) | | | |
| Platytheca micropora (lorica) | + | | |
| Amphimonas globosa | | | a |
| Amphimonas fusiformis | | | + |
| Spongomonas intestinum | | | + |
| Bodo alexeieffii | | | |
| Bodo angustus | | | |
| Bodo caudatus | + | | |
| Bodo cruzi | | | |
| Bodo edax | | | |
| Bodo glissans | + | | |
| Bodo globosus | | | |

---

a. Cells frequently in groups

| | Size Variation | Pigmentation | Colony |
|---|---|---|---|
| Bodo lens | | | |
| Bodo mutabilis | | | |
| Bodo minimus | | | |
| Bodo putrinus | | | |
| Dinomonas vorax | + | | |
| Rhyncomonas nasuta | | | |
| Bodopsis godboldi | | | |
| Pleuromonas jaculans | | | |
| Cercobodo crassicauda | + | | |
| Cercobodo radiatus | | | |
| Clautriavia parva | | | |
| Helkesimastix faecicola | | | |
| Dallingeria drysdali | | | |
| Tetramitus decissus | + | | |
| Tetramitus pyriformis | | | |
| Tetramitus sulcatus | + | | |
| Hexamitus inflata | + | | |
| Hexamitus pusillus | | | |
| Trepomonas agilis | + | | |
| Trepomonas rotans | | | |
| Trepomonas steinii | | | |
| 15 to 50μ | | | |
| Synura uvella | | Br | + |
| Dinobryon sertularia (lorica) | | Y | + |
| Monas vivipara | | | |
| Chilomonas paramecium | | | |
| Cryptomonas erosa | − | b | |
| Cryptomonas ovata | | b | |
| Cyathomonas truncata | − | | |
| Chlorogonium elongatum | | G | |
| Chlorogonium euchlorum | + | G | |
| Sphaerellopsis fluviatilis | − | | |
| Polytoma uvella | — | | |
| Carteria globosa | − | G | |
| Pyrobotrys gracilis | | G | + |
| Eudorina elegans | − | G | + |
| Euglena agilis | − | G | |

---

b. Olive-green to red

| | Size Variation | Pigmentation | Colony |
|---|---|---|---|
| Astasia inflata | + | | |
| Euglenopsis vorax | | | |
| Phacus stokesii | + | G | |
| Lepocinclis ovum | | G | |
| Strombomonas urceolata (lorica) | + | G-Br | |
| Rhabdomonas incurva | − | | |
| Menoidium pellucidum | + | | |
| Petalomonas carinata | | | |
| Petalomonas mediocanellata | | | |
| Petalomonas steinii | + | | |
| Entosiphon sulcatum | | | |
| Entosiphon ovatum | | | |
| Mastigamoeba limax | | | |
| Mastigamoeba longifilum | | | |
| Mastigamoeba sp. (Lackey) II | | | |
| Mastigamoeba chlorelligera | | c | |
| Mastigamoeba volutans | | | |
| Mastigella radiosa | | | |
| Mastigella sp. (Lackey) I | | | |
| Mastigella sp. (Lackey) III | | | |
| Poteriodendron petiolatum (lorica) | − | * | + |
| Bodo ovatus | | | |
| Bodo saltans | | | |
| Phylomitus amylophagus | | | |
| Cercobodo longicauda | − | | |
| Cercobodo ovatus | − | | |
| Tetramitus rostratus | − | | |
| Collodictyon triciliatum | | | |
| Hexamitus crassus | | | |
| Hexamitus fissus | | | |
| Hexamitus fusiformis | | | |
| Trigonomonas compressa | | | |
| 40 to 80$\mu$ | | | |
| Mallomonas caudata | | Y-Br | |
| Euglena gracilis | − | G | |
| Euglena polymorpha | + | G | |
| Euglena viridis | ± | G | |

---

c. May have a few pale green plastids

*Small chromoplasts may sometimes be seen

| | Size Variation | Pigmentation | Colony |
|---|---|---|---|
| Astasia klebsii | | | |
| Astasia quartana | | | |
| Astasia dangeardii | – | | |
| Phacus pyrum | | G | |
| Phacus pleuronectes | – | G | |
| Peranema trichophorum | ± | | |
| Heteronema acus | ± | | |
| Heteronema tremulum | – | | |
| Distigma proteus | ± | | |
| Anisonema truncatum | | | |
| Mastigamoeba sp. (Lackey) I | | | |
| Mastigella sp. (Lackey) II | | | |
| 60μ and greater | | | |
| Euglena acus | – | G | |
| Euglena deses | – | G | |
| Euglena fusca | | G | |
| Euglena mutabilis | | G | |
| Euglena oxyuris | | G | |
| Euglena tripteris | – | G | |
| Mastigamoeba trichophora | | G | |

# GLOSSARY

## A

*Algae*—Primitive and usually microscopic plants; in most cases containing chlorophyll and having a green, red, yellow or brown color. Many of these live in water. The chlorophyll bearing flagellates discussed in this book are considered by botanists to be algae but the group contains a great many nonmotile forms, as well as some colorless forms.

*Alveolar*—being made up of small cavities or chambers.

*Anastomosis*—the union or joining of two parts, usually leading to the formation of netlike structure.

*Anterior*—the forward (end).

*Apical*—on the anterior tip and in, or very near, the principal longitudinal axis.

*Aplanospore*—a nonmotile spore or cyst.

*Arborescent*—treelike

*Attenuated*—narrowed, tapered.

*Axial filament*—a reinforcing structure for a pseudopodium.

*Axopodium*—a pseudopodium with an axial filament.

*Axostyle*—a transparent rodlike structure which usually extends posteriorly from the blepharoplast along the major axis of the body.

## B

*Basal body*—synonym for basal granule.

*Basal granule*—a granulelike structure from which the flagellum arises.

*Biota*—the living organisms of an environment, both plant and animal.

*Blepharoplast*—the preferred scientific name for basal granule.

## C

*Catarobic*—the environment which has never experienced the effect of pollution.

*Cellulose*—the principal constituent of wood (plant cell walls).

*Chitin*—a horny substance which contains nitrogen and probably rarely occurs in protozoan shells (see tectin).

*Chlorophyll*—the green coloring matter of plants by which they convert carbon dioxide to food, utilizing light as an energy source.

*Chloroplast*—a plastid containing the green pigment chlorophyll.

*Chromatin*—the material stained by the so-called nuclear stains.

*Chromatophore*—literally color bearer. Usually synonymous with chloroplast.

*Chromoplast*—a color-bearing plastid; *e.g.*—chloroplasts are green chromoplasts.

*Colony, colonies*—groups of united cells.

*Conjugation*—the union of two cells (sexual reproduction).

*Contractile vacuole*—a cavity in the cell plasma having the property of contractibility.

*Cuticle*—the outermost covering—nonliving.

*Cyclosis*—circulation of protoplasm within the cell. Also called streaming.

*Cysts*—a resting cell, usually having a dense covering and, ordinarily, able to withstand adverse conditions.

*Cytopharynx*—a part of the cell mouth, located internally from the mouth proper. See also: gullet-reservoir.
*Cytoplasm*—the entire contents of the cytosome.
*Cytosome*—the region of the cell exclusive of the nucleus.
*Cytostome*—the cell mouth (rarely distinguishable in flagellates but ordinarily readily found in most ciliates).

### D

*Differentiation*—undergoing a change or producing a difference, or having division of labor.
*Distal*—most removed from the center of the body.
*Dorsal*—back (usually the part toward the observer in microscopic examination but sometimes otherwise—then the side opposite the ingestive area).

### E

*Ecology*—the study embracing the relation of an animal (or plant) to its surroundings.
*Ectoplasm*—the outer portion of the cytoplasm.
*Encyst or Encystment*—the process by which an active cell becomes a cyst.
*Endoplasm*—the inner portion of the cytoplasm.
*Endosome*—the central body of a vesicular type nucleus.
*Equatorially*—referring to middle region between the poles;i.e., between the anterior and posterior ends.
*Euglenoid*—having characteristics of the Euglenida.
*Excyst*—the process by which a cyst becomes an active cell.

### F

*Fauna*—the animals of an environment.
*Fibrilla*—a threadlike or fiberlike connective.
*Flora*—the plants (including bacteria) of an environment.

### G

*Gametes*—cells which fuse to form zygotes (at least somewhat analogous to the egg and sperm cells).
*Glycogen*—animal starch; gives reddish-brown color with iodine.
*Gullet*—a modified mouth or portion thereof.
*Gullet-reservoir*—the flask-shaped anterior invagination associated with euglenoids—sometimes called cytopharynx.

### H

*Heterogamy*—conjugation of cells noticeably different in size.
*Holophytic*—entirely plantlike in nutrition.
*Holozoic*—entirely animallike in nutrition—ingesting particulate food.
*Hyaline*—colorless or transparent.

### I

*Imbricated*—with scales which are overlapping.
*Ingest*—to take in food.
*Ingestive area*—region of body where food is taken in (the distinguishable mouth

in many protozoa).

*Isogamates*—gamates which appear identical (in size, shape, etc.) but are capable of uniting to produce a zygote.

K

*Karyosome*—condensed masses of chromatin, or large knots on the chromatin net of the nucleus.

*Kinetic element*—cell parts which are active in the division of the cell (may include nucleus, blepharoplast, centriole, rhizoplasts, parabasal bodies and others).

*Kinetonucleus*—a structure consisting of blepharoplast and parabasal body.

L

*Lateral*—pertaining to the side.

*Leucosin* (more recently named "chrysose")—a carbohydrate stored as food by some protozoa (especially chrysomonads).

*Littoral*—pertaining to the shore.

*Lorica* (pl. *Loricae*)—a shell or other covering structure which certain protozoa (and metazoa) construct. (Loricated—having a lorica.)

*Lumen*—a central cavity or something having the appearance of a central cavity.

M

*Macro*—a prefix meaning large.

*Matrix*—the lifeless matter between cells.

*Mesosaprobic*—the environment in which oxygen is increasing rather than decreasing, turbid to clear, ammonia being oxidized, hydrogen sulfide does not persist.

*Metaboly*—the type of motion exhibited by the euglenoids.

*Metazoa*—the multicelled animals having tissues and usually also organ structures.

*Micro*—a prefix meaning small.

*Monad*—small (usually less than $20\mu$) mona- and bi-flagellated organisms.

*Morphology*—the study of shapes of organisms.

N

*Nucleolus*—a distinct body within the nucleus consisting of plastin (therefore also known as a plasmosome) which does not take on the usual nuclear stains.

*Nucleus*—the life center of the cell, often identified by its ability to be colored by certain stains.

O

*Oligosaprobic*—the environment which is relatively stable and practically saturated with oxygen.

*Organism*—individual plant or animal in the living state.

P

*Palmelloid state*—cells with a gelatinous covering which is confluent between cells. Cells lack definite orientation. Masses of cells in this state are spoken of as *palmellae* (sing. *palmella*).

*Papilla*—a nipplelike projection.

*Parabasal body*—a body associated with the blepharoplast that stains with nuclear stains. It is ordinarily several times to many times larger than the blepharoplast.

*Paramylum*—a carbohydrate food reserve stored by euglenoids.

*Parthenogenesis*—development of female gametes without fertilization.

*Pectin*—a jellylike material, chiefly carbohydrate in composition.

*Pelagic*—pertaining to open water.

*Pellicle*—a delicate superficial membrane.

*Pellicular*—having to do with the pellicle.

*Peripheral*—located adjacent to the outside.

*Periplast*—practically synonymous with pellicle.

*Peristome*—the region surrounding the mouth.

*Pharyngeal rods*—trichites or similar structures extending inward from the mouth proper.

*Planktonic*—referring to organisms, both plant and animal, which float freely or swim in open water.

*Plastid*—formed bodies in the cells.

*Polysaprobic*—the environment in which active decomposition is taking place. Characterized by little or no dissolved oxygen, the frequent presence of hydrogen sulfide, nitrogen in the form of ammonia, and distinct, usually gray, turbidity.

*Posterior*—the rear (opposite of anterior).

*Protoplasm*—the entire material of the living cell.

*Protoplasmic*—pertaining to the protoplasm.

*Protoplast*—a cell as distinguished from cell wall, lorica or test as a part of the organism.

*Proximal*—part nearest center of the body (opposite of distal).

*Pseudopodium*, (pl. *Pseudopodia*)—protoplasmic extensions.

*Punctae*—raised, dotlike markings.

*Pyrenoid*—a distinct body within the chloroplast. It is apparently the center of carbohydrate (starch, paramylum, leucosin) production.

*Pyriform*—candle flame shape (see footnote page 21).

### R

*Radial symmetry*—symmetry in which parts opposite each other on an axis are similar.

*Rhizoid*—rootlike.

*Rhizoplast*—a fibrilla connecting the blepharoplast to the nucleus.

*Rhizopodia*—a rootlike (branched) pseudopodia.

*Rhizostyle*—a stiff, reinforcing rhizoplast.

*Rostrum*—a square projection of the anterior.

### S

*Sapropelic*—a term usually applied to faculative or obligate saprozoic ciliates and usually anaerobic forms such as those found in detritus on stream bottoms.

*Saprophytic*—living on dead or decaying material.

*Saprozoic*—similar to above—animal living on soluble substances from decaying material.

*Stigma*—a red, orange, or otherwise colored, pigmented structure found in many flagellates—also called eyespots.

*Stoma*—mouth.

*Stria* (pl. *Striae*)—longitudinal marking of the pellicle, ectoplasm or other external layer.

*Substrate*—the surface on which an organism moves or, also, the medium in which it lives.

## T

*Tectin* (Pseudo-chitin)—a mucinlike substance apparently capable of hardening, by deposition within itself, of calcium carbonate or by inclusion of sand or other foreign materials.

*Trichites*—a fine rod- or needlelike protrusible structure, thought to originate from trichocysts.

*Trichocyst*—a fine rod- or needlelike protrusible structure.

*Truncated*—cut off.

## V

*Ventral*—bottom side or side away from the observer—usually side with mouth opening.

*Vesicle*—any small bladderlike cavity.

*Vesicular*—the term applied to the usual flagellate nucleus, which consists of a dense central body (endosome) surrounded by nuclear sap which is in turn enclosed by the nuclear membrane.

*Vibratile*—capable of vibration.

*Volutin*—a proteinlike stored food material—stains with hemotoxylin but destains readily.

## Z

*Zygote*—cell formed by the union of two gametes.

*Zygospore*—an encysted zygote.

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